CAISSON DISEASE OF BONE - AN EXPERIMENTAL STUDY OF ITS CAUSE, EARLY DIAGNOSIS, AND MANAGEMENT.

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CONTENTS.

ABSTRACT

The aetiology of caisson disease of bone remains incompletely understood. Re-implantation of anoxic autologous marrow into rabbit femora can produce a 'lesion' with a surrounding ring of fibrous tissue and bone and this may mean that any factor causing an area of marrow anoxia or necrosis could lead to the development of a lesion resembling that seen in caisson disease of bone.

Six Göttingen miniature swine underwent multiple hyperbaric exposures and a number of possible indicators of the early stages of osteonecrosis were recorded. None developed lesions resembling caisson disease of bone though two developed aeroembolism of the marrow cavities of long bones. Regular serum samples were obtained from indwelling silastic right atrial catheters which remained patent for up to twelve weeks.

Osteonecrosis was produced artificially in rabbits by arteriolar blockade and an earlier report of positive scintigraphy in relation to this was confirmed. However, measurements of collagenolytic enzymes (measured as serum proline imino-peptidase activity), did not show any statistically significant alteration and this measurement may therefore not be useful as an early indicator of caisson disease of bone. Skeletal scintigraphy of single joints in divers is reported and two diphosphonate skeletal imaging agents (MDP and EHDP) compared.

Because of uncertainty about the factors determining the localisation of skeletal imaging agents in areas of osteonecrosis a technique was developed for the microautoradiographic localisation of these agents in undecalcified cancellous bone. Early results of these studies indicate that the enhanced concentration of skeletal

imaging agents in areas of new bone formation may be a result of an increase in their uptake by the bone cells.

The possible management of caisson disease of bone is briefly discussed and a recommendation made for reconsideration of the role of forâge- biopsy in any surgical management of juxta-articular lesions.

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SECTION ONE

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INTRODUCTION

CHAPTER 1 - BACKGROUND INFORMATION

DEFINITION OF TERMS

The term caisson disease of bone is applied to areas of bone necrosis (or 'dead bone') discovered after a person has been subjected to increased ambient pressure. Exposure to such a 'hyperbaric environment' produces various effects upon the body, and several of these may only become evident during, or shortly after, the return to atmospheric pressure. Caisson disease of the bone usually makes its presence known if collapse or fragmentation of the necrotic bone occurs in a site where this causes irregularity of a neighbouring joint surface. Consequently, it tends to present clinically months or even years after the causative hyperbaric exposure, and is therefore frequently not related to it either by the sufferer or the examining doctor.

The term caisson disease of the bone was first adopted for this condition because it occurred in men who work in caissons constructing the foundations of bridges. However, this construction method, using increased air pressure to stop the work site flooding, had been used for many years before it was recognised as a cause of bone necrosis similar to that occurring in the absence of hyperbaric exposure, as, for example, in persons with sickle cell anaemia. This descriptive name, indicating the occupational origin, was used for many years, but more recently attempts at fitting caisson disease of the bone into present day pattern of nomenclature have produced a variety of synonyms. The current term for areas of dead bone, as found in several circumstances including caisson disease, is <u>osteonecrosis</u>, which does not imply any particular aetiology but just means 'dead bone'. A number of other terms have also been used, e.g. aseptic,

avascular, or ischaemic necrosis of bone. As there are other causes of macroscopically and miscroscopically similar lesions the term osteonecrosis must be qualified by some word to indicate that the harmful agent is thought to be an exposure to pressure. The word 'hyperbaric' is not favoured because it is not thought that pressure in itself is harmful but rather that changes in pressure are, and therefore the word 'dysbaric' has recently been suggested. The reason for this is given by Beckman and Elliott (1974) in the introduction to the first symposium devoted to this disease:-

'The term is chosen carefully so that both hypobaric and hyperbaric workers may be included. This usage precludes scientific prejudgement regarding whether the etiological factors are associated with compression, exposure, decompression, or any other features unique to these occupations.'

However, 'dysbaric osteonecrosis' or 'dysbarism-related osteonecrosis' is not a readily translatable term and there is much that could be said for retaining the internationally recognised and understood term caisson disease of the bone at present.

The reason why the terms avascular necrosis and ischaemic necrosis may particularly not be appropriate in caisson disease of the bone will become evident in the discussion of postulated aetiology in Chapter 2. These terms may of course still be appropriate for the osteonecrosis which develops following interruption of bone blood supply by fracture (e.g. subcapital fracture of the femoral neck, fracture of the waist of the carpal scaphoid, or fracture of the neck of the talus) or dislocation (e.g. dislocation of the lunate bone or the femoral head after hip dislocation).

Osteonecrosis is also recognised as occurring without preceding trauma or hyperbaric exposure, especially in the femoral head (unilaterally or bilaterally) and this has been reported as being associated with:-

Excessive alcohol consumption

Hepatic disease

Gout or hyperuricaemia

Hypercholesteraemia

Hyperlipidaemia (particularly Type IV - associated with alcoholism)

Alkaptonuria

Gaucher's disease

Polyarteritis nodosa

Systemic lupus erythematosis

Pancreatitis (acute or chronic) (only reported in people who were also alcoholic)

Haemoglobinopathies (Sickle cell disease and sickle cell thalassaemia)

Steroid therapy (especially in renal transplant recipients)

Diabetes

Finally, many instances of osteonecrosis with no known or postulated aetiological factor have been described and the term 'idiopathic' has been applied to them. For review articles on osteonecrosis of other aetiologies see Zinn (1971) and the Proceedings of the Conference on Aseptic Necrosis of the Femoral Head (1964)

The evidence for the association of osteonecrosis with excessive alcohol consumption has been seriously questioned on the grounds that it underestimates the alcohol consumption of the

control population without osteonecrosis (Zinn, 1971; Walder, 1974 a). Some of the other proposed aetiological factors appear to be inter-related and while some clearly could affect the circulation to the bone in others the reasoning is more devious.

HISTORICAL INTRODUCTION

Landmarks in the recording of observations of the effects of decompression will be briefly mentioned here, but no attempt will be made to summarise the history of compressed air work or diving.

The first account of acute decompression sickness appears to be that of Pol and Watelle (1854). According to Paul Bert, writing in 1878, the acute effects noted during or shortly after decompression from excess pressure included respiratory difficulties; alterations of pulse rate and volume; muscular pains (noted to be nearly always the first symptom to present); skin itching; subcutaneous emphysema; cerebral symptoms including loss of consciousness; deafness and blindness; paralysis; and sudden death Autopsy findings on patients who died later of complications of paralysis showed 'white softening' of lengths of spinal cord. Autopsy on two cases of sudden death showed congestion of the lungs, liver, spleen, and kidney but no abnormality in the central nervous system.

To the available clinical details, Bert added a great deal of experimental work in a variety of animals. This work included blood gas analyses on animals in a hyperbaric environment, the effects of increasing oxygen tension and the description of oxygen toxicity; the effects of rapid decompression from excess pressure, including analysis of intravascular gas bubbles (which he found to be mainly nitrogen) and noting the presence of bubbles in the

subcutaneous tissues, cerebro-spinal fluid, and anterior chamber of the eye. The last of these had been previously noted in the eye of a viper during decompression in an 'exhausted receiver' i.e. to sub-atmospheric pressure, by Robert Boyle (1670). Bert also noted gas bubbles in the circulation of living animals by decompression from excess pressure of transparent young eels. He attempted recompression treatment of animals and treatment with pure oxygen to increase the diffusion gradient of nitrogen from the pulmonary capillaries to the alveolae and he recommended controlled slow decompression to prevent symptoms. The importance of Bert's experiments and the conclusions to which they led him are shown best by the need to translate his work into English in the midst of the second world war (actually for his work on altitude physiology more than for that on increased pressure).

Other researchers came to the same conclusion that controlled slow decompression would prevent symptoms of acute decompression sickness, by allowing excess gas to diffuse out of the body (via the lungs) along pressure gradients as the pressure dropped rather than to build up and give rise to supersaturation of the tissues and consequently risk bubble formation. The first recommended schedules for safe decompressions following exposure of known duration to given pressures were the so called stage decompression stables of Boycott et al. (1908). In these schedules the raised exposure pressure is dropped to atmospheric pressure in a number of steps at each of which a stop is made for some stated time. Hill (1912) produced the first safe decompression schedules using a continuous decompression profile.

Both these methods of decompression have persisted over the years though the tables have steadily been extended as experience

has accrued and there has been increasing use of stage decompression rather than a continuous reduction of pressure.

Bone changes were first attributed to hyperbaric exposure by Twynan in 1888 in his report of 'a case of caisson disease' but this was an infected lesion and is now regarded as being an account of chronic osteomyelitis (McCallum et al., 1966; Ohta and Matsunaga, 1976; Davidson, 1976a). There have been no further instances reported linking osteomyelitis with hyperbaric exposure and this would appear to have been a chance association.

The first reports of the osteonecrotic lesions now regarded as the characteristic bone changes found following exposure to excess pressure were the clinical and radiographic reports of Bornstein and Plate (1911) and Bassoe (1913). Bornstein and Plate noted three cases of bone necrosis, one having a right femoral head 'shaped like a potato' with osteoarthritis, the second having in the right shoulder an 'egg shaped' condyle with obvious radiographic density and osteoarthritis, and a third with both femoral heads deformed and arthritis in both hip joints. Bassoe described similar lesions but in addition noted a lesion in the proximal shaft of a tibia which he recorded as:- 'two inches below the left knee joint there are changes in the spongy tissue of the tibia in the shape of irregularly branching or stellate islands of sclerosis'. In their conclusions Bornstein and Plate felt that the lesions were found in the condyles because function was affected whereas bone atrophy in the shaft would have no serious effect. Alternatively, they argued that the blood supply of the epiphysis of long bones might be worse than that of the shaft so that gas bubbles formed preferentially at these sites during decrompression. In view of the disability of their patients they

also recommended that joint radiographs be recorded at pre-employment medical examinations in case of subsequent workmens' compensation claims. This is an interesting foresight. Compensation claims now mean that insurance rates in the United States for compressed air work can be $42.00 per $100 payroll (Johnson, 1974).

Many reports have followed these and according to Davidson (1976a) there are over one hundred and thirty published reports of the radiographic appearances of caisson disease of bone. The majority of these refer to compressed air workers though all the typical lesions of dysbaric osteonecrosis are now recognised as also occurring in divers. The first report of a bone lesion in a diver is quoted by Elliott and Harrison (1971) as that of Grutzmacher (1941) but they refer to an earlier account by Seifert (1936) of a man who had worked on the sea bed within a diving bell.

Decompression to subatmospheric pressures, such as occurs in balloonists and aviators, appear to have produced very few documented instances of osteonecrosis. The most convincing report is that of Fryer (1969) of a lesion affecting a humeral head, which is a site rarely affected by osteonecrosis of other aetiologies, in a photo survey pilot. A report of bilateral hip joint changes is made by Hodgson et al. (1968) and another by Markham (1967) though this latter report could have alternative causation as the man was diabetic and had also had 'local parenteral steroid therapy'. Surveys of men exposed to simulated altitude (low pressure chamber operations) have revealed an intraedullary infarct in the mid shaft of a femur and a round radiolucent area in the neck of a humerus (Hodgson, et al. 1968). These were the only two cases found in 164 men with an average exposure time to altitudes of 20,000 feet

of 169 hours per man, so the evidence of osteonecrosis attributable to decompression to subatmospheric pressures appears to be much less than that of hyperbaric exposure (either diving or compressed air work).

The next landmark in recording observations of effects of hyperbaric exposure is the work of Rozsahegyi. He performed detailed follow-up examinations of men involved in compressed air work during construction of the Budapest underground. This examination included electroencephalography (Rózsahegyi and Roth, 1966). Abnormalities were found in two thirds of a group of 57 workers who had suffered once or several times from acute forms of central nervous system decompression sickness, compared with a 20% incidence recorded in the same publication of a small group of workers who had never been affected by decompression sickness. Seven of the fifty-seven workers had suffered as their acute form of central nervous system decompression sickness paraplegia without loss of consciousness, yet three of these had abnormal EEG records. While these numbers are too small to be of any statistical significance, they raise the possibility of brain damage occurring asymptomically in these circumstances, and the authors felt that central nervous system decompression sickness lesions must always be considered multifocal. In support of this they state that many of the electroencephalograph tracings showed diffuse abnormalities.

Further reports along these lines would be very useful but with increasing strictness in adherence to decompression schedules, and the general lengthening of decompression times since the 1950's, central nervous system decompression sickness is becoming relatively uncommon and further reports are likely

to be restricted to animals. In a study of autopsy material from forty four goats subjected to various decompression procedures no brain lesions were found despite focal infarction of spinal cord white matter in twenty four animals, (Palmer et al., 1976).

It is interesting to note that the old German decompression tables used during the Budapest underground construction required a decompression time of twenty six minutes after a six hour shift at 25 p.s.i.g. (pounds per square inch guage pressure)whereas the present United Kingdom (Blackpool) tables would require ninety minutes, and the Washington State tables (U.S.A.) one hundred and twenty two minutes. This together with therapy by early recompression, assuming virtually all symptoms arising within 24 hours of decompression to be decompression sickness, has led to a decreased incidence of central nervous system lesions. This means that caisson disease of bone, which occurs asymptomatically and is therefore not treated at the time by recompression, assumes relatively greater importance.

To quote Davidson and Griffiths, writing in 1970:-
'Although decompression sickness has not yet been completely eliminated, a well organised medical service, and careful recompression treatment can enable the situation to be well controlled. This is not so for the long term effect, caisson disease of bone, which is therefore now considered to be the major hazard of working in compressed air'. Since 1970 the world in general, and countries bordering the North Sea in particular, have seen a great increase in deep sea diving acitivity associated with oil extraction from sub-sea areas of continental shelf. It seems fair to say that this quotation could now be

extended to include divers as well as compressed air workers.

TYPES OF DECOMPRESSION SICKNESS

Acute decompression sickness may show itself in a variety of ways. The commonest is pain localised in a limb, type 1 decompression sickness, colloquially known as 'the bends'. Other manifestations are potentially more serious and are called type 2 decompression sickness. The distinction was first suggested by Golding et al. (1960).

Before discussing the present classification it should be noted that it is most unusual for decompression sickness to result from an exposure to a pressure of not greater than two atmospheres (equivalent to a depth of ten metres of sea water). Present decompression tables for exposure in compressed air workings to pressures greater than this are calculated in the hope that the overall incidence of acute decompression sickness (the so called 'bends rate') will be kept to about one per cent of man decompressions. In the past diving decompression tables have always been claimed to be more conservative. Individuals vary in their susceptibility to decompression sickness and in most contracts, it has been found that about one third of the exposed men account for three quarters of the cases of decompression sickness requiring therapeutic recompression (Sealey, 1967). In a particular individual it has been felt that departure from normal resistance to decompression sickness may occur in association with additional stress factors such as fatigue, heavy exercise, hypoglycaemia, and anxiety (Kidd and Elliott, 1975) but this is unproven at present.

Type 1 Decompression Sickness.

Most attacks of acute decompression sickness fall into

this group. The first symptom complained of is usually pain, but may be skin irration or localised swelling. The symptom may start during the final stages of decompression from excess pressure or up to 24 hours after the completion of decompression. Most cases arise within one hour of the completion of decompression (Kidd and Elliott, 1975).

Musculo-skeletal ('the bends'): This starts with pain, often in a limb, and usually in the region of a major joint. In compressed air workers, the lower limbs are most commonly affected (Griffiths, 1975) whereas in divers the upper limb is the site of predilection (Kidd and Elliott, 1975), although this may not be so following saturation dives. The pain may not be severe, and the popular term for a minor degree of discomfort is 'the niggles'. On the other hand, the pain may be severe, but in either case there is no constitutional upset, in contrast to type 2 decompression sickness. Recompression usually brings rapid relief, but there are exceptions. Occasionally pain is made worse by recompression and repeating the recompression at a slower rate of increase of pressure is then necessary (Griffiths, 1975).

Sometimes gas within or around a joint may produce a squelching noise on movement without any accompanying pain. This disappears after a few hours without recompression treatment (Griffiths, 1975)

Cutaneous : The skin is affected by a number of minor symptoms. There may be pruritis, especially during decompression. This is commonly referred to as 'divers fleas'. It tends to be self-limiting but sometimes presages the development of areas of cutaneous vasodilatation leading to stasis of blood and a bluish mottled appearance. This is colloquially termed 'bruising' or 'staining' and is variously described as most commonly occurring over the trunk or shoulder regions.

Lymphatic: This form consists of a localised swelling, rather sore and tender when examined. It may be associated with an area of musculo-skeletal type 'bends' pain but it may occur alone. The usual site is the neck and shoulder regions. When examined there may be evidence of subcutaneous gas and distal pitting oedema suggestive of lymphatic obstruction. The response to recompression is often satisfactory.

Type 2 Decompression Sickness

This is more serious than the above. The onset is sooner after reaching atmospheric pressure and the great majority begin within forty five minutes of the end of decompression (Griffiths, 1975). In diving practice many type 2 cases develop during decompression, i.e. before the surface is reached. Constitutional symptoms are the rule and the person affected often feels and looks ill. Virtually any symptom occurring within 24 hours of hyperbaric exposure must be assumed to be due to decompression sickness because very many forms of atypical onset (which have responded well to therapeutic recompression) have been described. Despite this, there are certain common manners of onset affecting the nervous, respiratory and cardio-vascular systems.

Neurological system : This is the system most often involved in type 2 decompression sickness. It may be preceded or accompanied by respiratory system or musculo-skeletal (type 1) decompression sickness. The spinal cord is involved more often than the brain. Peripheral sensory and motor lesions may initially be dismissed by the person affected as 'pins and needles' but symptoms of parasthesia are very important. The affected individual may rapidly develop motor weakness to the extent of paraplegia and associated sensory progression may include loss of sensation of bladder and bowel control. Stomach pain is less common a symptom and may be associated with nausea and

vomiting.

More dramatic onset may take the form of sudden collapse, with or without loss of consciousness, and cerebral symptoms other than coma include dizziness with vertigo and nystagmus (a 'labyrinthrine bend' or 'the staggers'; not to be mistaken for drunkenness), tinnitus, partial deafness, migraine-like symptoms, eye signs such as scotomata and fortification spectra, and even mimicing of psychotic conditions. 'Headache is an important symptom that must not be ignored' (Griffiths, 1975).

It must be added that musculo-skeletal type pain in a 'girdle' distribution on the trunk also denotes central nervous system involvement, and must be treated as type 2 decompression sickness and not type 1.

Respiratory system : The colloquial term 'the chokes' aptly describes the common manner of onset. There is sub-sternal or thoracic discomfort on inspiration, causing reflex coughing.

Cardio-vascular system : Haemoconcentration due to the leakage of plasma into the extra-vascular space may lead to signs of hypovolaemic shock. The description of praecordial 'tightness' associated with fatigue and signs of shock may mimic myocardial infaction.

The response of type 2 decompression sickness to emergency therapeutic recompression is nearly always dramatic and the relief of symptoms is usually rapid. This is useful as anyone with unusual symptoms which present within 24 hours of decompression can be subjected to recompression, which is unlikely to do any harm, as a diagnostic manoeuvre. If the symptom is relieved then the diagnosis is decompression sickness and a slow decompression on one of the appropriate therapeutic tables can follow. Symptoms are more likely to be relieved if recompression is a short time interval after their

onset, but complete relief can occur even after a considerable delay, though a greater recompression pressure may be needed. Because haemoconcentration is often present, in addition to therapeutic recompression intravenous low molecular weight dextran is usually advised as a plasma volume expander.

LONG TERM SEQUELAE

Two chronic sequelae have been attributed to decompression. The first of these is caisson disease of bone. This is to be described in detail in the following section on its pathology.

The second is central nervous system damage. This includes cases of paraplegia following a spinal cord lesion. Whilst therapeutic recompression within a few hours or occasionally days of the onset will often result in almost complete recovery, a small number of men are left with residual muscle weakness, patches of numbness, or difficulties with bladder control. These usually continue to improve without further treatment for some months, but Kidd and Elliott (1975) regarded any lesions remaining six months after the acute episode as permanent.

Permanent vestibular damage following a 'labyrinthine bend' has been reported by Leitch (1971) and a number of less specific defects including dizziness, impotence, and pathological change of personality were reported by Rózsahegyi (1959).

HISTO-PATHOLOGY OF CAISSON DISEASE OF BONE

The histo-pathology of dysbarism-related osteonecrosis does not appear to differ, on the small amount of material examined in detail, from osteonecrosis of other aetiologies. The only literature giving full histological descriptions of caisson disease of bone are the reports of Kahlstrom et al. (1939), McCallum et al. (1966), and Weatherley et al. (1977b).

The early course of events leading to the described histological findings is not definitely known. If the lesions are caused by an interruption of the arterial blood supply it may well be that the juxta-articular lesions of caisson disease of bone affecting the humeral and femoral heads develop in a similar manner to those seen in the necrotic femoral head after high transcervical (subcapital) fractures of the femoral neck. Such femoral heads are often removed early and have been studied on a day-to-day basis by Catto (1965). The first histological changes she reported were of 'agglomeration' and necrosis in the haemopoietic marrow, even preceding the loss of osteocytes from the bone. In fact it was not until three weeks or more after the fracture that empty lacunae could be found throughout the area of necrosis. Catto described also the histological features of revascularisation of dead bone and marrow and noted the laying down of new bone on dead trabeculae. She made the point, which is particularly relevant to caisson disease of bone, that necrotic bone shows no alteration in radiological density and that if radiographic examination showed increased radio-opacity this was because of new bone formation on dead trabeculae leading to an absolute increase of bone mineral per unit volume of cancellous bone.

The histological examination of human bones affected by caisson disease is limited to specimens which were from areas of increased radio-opacity on radiographic examinations. The areas of dead bone are recognised histologically by the absence of osteocytes in the lacunae and by a corresponding loss of cellularity of the bone marrow. Perhaps surprisingly, of more interest is the examination of surrounding living bone marrow, which is found to contain dead trabeculae 'entombed' within the living bone. The area of occurrence of these entombed trabeculae can be mapped out and taken to indicate the area

of osteonecrosis originally present, before revascularisation and repair took place. That this assumption is valid has not always been accepted, though Kahlstrom et al.(1939) stated that it seemed to be the best explanation. The work of Catto (1965), among others, in following the revascularisation of femoral heads after fracture, shows this process occurring so clearly that it is now difficult to think of any alternative hypothesis. These dead trabeculae remain and can be used as a 'marker' for mapping out the original necrotic area for a considerable time; in the case reported by Weatherley et al. (1977b) the period of compressed air work had ended eleven years before the man died from an unassociated cause.

Of especial interest is the junctional zone between dead bone and the area which has been dead but which has been repaired. Examination of this not only might give information on the sequence of the repair process but also might indicate why it had ceased at that place and why the entire lesion had not been repaired. The established junctional zone seems to be similarly described in all published account. That of Weatherley et al. (1977b) points out that the radiological appearance of the lesions in their subject was known not to have changed in the preceding four years.

The zone will be described in the direction in which it is repairing. The dead trabeculae are surrounded by new bone, until they become so thick that the intervening marrow spaces are occluded and the trabeculae become continuous one with another in a series of arches. Beyond this particularly thick layer of viable bone is some calcification present as small granules and as larger dystrophic masses, this area also shows some fibres of collagen and reticulin. Beyond this again is a band of dense fibrous tissue. The thick layer of bone calcification in the junctional zone corresponds to the dense

line seen on radiographs and known as 'linear opacity'. Thus, the presence of a 'linear opacity' on radiographic examination might be taken to indicate the presence of a junctional zone and that no further repair of that particular lesion will take place.

The reason for this arrangement of the junctional zone is not clear. A hypothesis might be proposed that the revascularising arterial supply 'could not stretch any further' and the vessels stop regenerating; this being the initial event deciding the place at which repair stops. A more plausible way of saying essentially the same thing is that the end arteries, carrying oxygenated blood to the regenerating tissues, can only supply a limited amount of active bone-forming and marrow-forming tissue before the oxygen tension becomes decreased, so much that further repair is not possible.

It is here relevant to mention the work of Bassett (1962). He used 'primitive fibroblasts' taken from bone in tissue culture experiments. He subjected these cells to different environmental conditions and showed (a) development into fibrous tissue in conditions of mechanical tension and high oxygen levels; (b) development into cartilage in conditions of compression and low oxygen levels; and (c) development into bone in conditions of compression and high oxygen levels. Unfortunately, this work does not fit with the above hypothesis for the following reasons: firstly, more bone is formed at the limit of the repair where the hypothesis predicts the available oxygen will be less, and secondly, there is a band of dense fibrous tissue when the mechanical forces within the intact bone will be compressive. However, it should be made clear that Bassett himself regarded the 'environmental conditions' to be dependent also upon products released by the cells themselves as well as upon tissue structure.

An alternative hypothesis invokes the mechanical aspects of bone repair process. Weatherley et al. (1977b) proposed that the arches of bone joining the trabeculae arose for structural support. They felt that as the repairing lesions are asymptomatic the subject maintains his usual activity, and bones affected with osteonecrosis would be subjected to full load-bearing. The series of arches seen histologically would be the arrangement giving best structural support to the remaining dead bone. Similar organisation is evident in histological sections of subchondral bone, and the importance of this arrangement for the resistance to a compression force has been measured in rabbit femoral heads by Szepesi (1978).

Neither of these hypotheses appears to provide a complete explanation of the observed changes. Furthermore, the results of ancillary investigations have not helped. The above account of the junctional zone is based on specimens from a supposedly stable situation where the bone lesion has remained incompletely healed. However, Gregg (1977) found that lesions which had shown no change on radiographic examination for ten years could still be associated with an increased uptake of skeletal imaging agents on scintigraphic examination. Thus it seems that the turnover of hydroxyapatite is still greater than normal in the affected area and it may be that the situation is not as stable as it appears and the potential to complete the repair is present if it could be released.

Further changes in osteonecrosis can develop in lesions involving a joint surface. The articular cartilage can become replaced to a variable extent by fibrocartilage. The necrotic bone may collapse or segments separate. Such segments usually include the overlying cartilage. Either of these developments will lead to irregularity of the normal contour of the articular

surface. Separated fragments become osteocartilaginous loose bodies within the joint, and section of one of these present for many years showed a centre of calcified fibro-cartilage and outer layer of irregularly laminated fibro-cartilage and calcified cartilage (Kahlstrom et al., 1939). The development of degenerative arthritis can lead to:-

erosion of cartilage and the articulating surface becoming exposed bone; the evolution of subchondral cavities filled with fibrous tissue or coagulum; osteophyte formation; and associated changes in the opposing joint surface.

Shaft lesions appear macroscopically to consist of necrotic greyish friable material surrounded by a shell of bone. Histologically, the bony shell is a junctional zone as already described. The greyish material is necrotic marrow, sometimes containing calcareous granules and sometimes the well preserved outlines of dead fat cells can be seen. Although without nuclei these cells could be shown still to contain fat when suitable staining was used (Weatherley et al., 1977b). Shaft lesions appear to remain static once they have been 'encapsulated' by a wall of bone and fibrous tissue, but some still show increased uptake of diphosphonate imaging agents on scintigraphy many years later (Gregg, 1977). The interest in this finding in shaft lesions is that in juxta-articular lesions it could be objected that positive scintigraphy is attributable to early osteoarthritic changes (even if not yet visible radiographically) but this cannot be the case in shaft lesions. Another piece of indirect evidence indicating continuing activity in shaft lesions is that there are now reports of three sarcomata arising in association with longstanding shaft lesions. The first was reported as an anaplastic fibrosarcoma (Dorfmann

et al., 1966) and the other two as milignant fibrous histiocytomas (Mirra et al., 1974). The minimum period from the end of compressed air exposure to the development of a sarcoma was seventeen years. Occasional sarcomata have also been reported arising in association with long standing bone infarcts not related to caisson disease. Many large mononuclear cells of uncertain identity were seen around a femoral shaft lesion by Weatherley et al.(1977b), but their significance is uncertain.

In summary, the histopathology of caisson disease of bone is poorly documented, but important questions are posed by the partial repair of lesions. The first of these is that if small animals are compressed and decompressed, lesions may be produced which are not detected on later macroscopic or radiological examination because they have completely repaired by virtue of their small size. The second is to determine what causes repair to cease so that treatment might be instituted to stimulate the remaining necrotic area to repair before it underwent collapse of fragmentation. It is not at present clear whether there would be any advantage in giving treatment before the boundary zone forms even if it were possible to detect the lesions at that stage. Radiological screening often first detects a linear opacity which may be interpreted as showing that the repair process has already ceased. It is possible that by radionuclide skeletal scintigraphy, which detects an increased rate of new bone formation, lesions may be detected earlier, and this will be discussed in detail in Chapter 3.

PRESENT CLASSIFICATION OF CAISSON DISEASE OF BONE

For ease of description the radiological appearances of caisson disease of bone have been classified. The first detailed scheme appears to be that proposed by Bell et al. (1942). They

gave three graduations of shaft lesions (+ ++ +++) and four graduations of epiphyseal lesions. The scheme widely adopted in the English literature at present is that proposed by the Medical Research Council Decompression Sickness Panel (McCallum et al., 1966) and this is reproduced below. From time to time other authors (e.g. Ohta and Matsunaga, 1974) have suggested minor modifications.

Medical Research Council Decompression Sickness Panel Classification of Bone Necrosis in Compressed Air Workers and Divers

JUXTA - ARTICULAR LESIONS

A1. Dense areas, with intact articular cortex

A2. Spherical segmental opacities

A3. Linear opacity

A4. Structural failures

a. Translucent subcortical band

b. Collapse of Articular cortex

c. Sequestration of cortex

A.5 Secondary degenerative arthritis (osteoarthritis)

HEAD, NECK AND SHAFT LESIONS

B1 Dense area (not bone islands)

B2 Irregular calcified areas

B3 Translucent areas

B4 Cortical thickening

For specimen radiographs of each of these types see Walder (1974b).

This classification does not include bone islands as these are not felt to represent a form of dysbarism-related bone lesion. This is not universally agreed, although a careful study using a control group of non-divers and three radiologists reading the radiographs independently showed that bone islands occur as

frequently in the control population as in divers (Davidson et al., 1977). The same study reports that histological examination of bone islands did not show any bone necrosis. The 'cysts' found in certain sites, especially in the femoral neck, and 'geodes bordet', were also found by this study to be no more common in divers.

The increasing number of radiographic records held by the Medical Research Council Decompression Sickness Central Registry in Newcastle-upon-Tyne has necessitated the transfer of records to computer storage for ease of access and analysis. The above classification scheme is not in a suitable form for input into a computer, and the information is, therefore, processed as follows:-

For each of eight sites (right and left proximal humeri and femora, distal femora, and proximal tibiae) a letter is recorded as a direct translation of the radiologists report. For example,

C = Negative (normal)

Radiologists report juxta-articular lesion:

P = suspected

Q = definite A1

R = definite A2

S = definite A3

T = structural failure A4a

U = structural failure A4b

W = structural failure A4c

X = A5

Z = operation

Radiologists report neck and shaft lesion:

J = suspected

K = definite B1

L = definite B2

M = definite B3

N = definite B4

Radiologists report other unrelated abnormality

E = Bone island

F = Cyst in neck of femur or upper humerus

G = Other unrelated abnormality

This information is summarised for each man by a number denoting the potentially most serious lesion present;

0 = all sites normal (letter C)

1 = No decision

2 = Irrelevant abnormalities (E, F or G)

3 = Suspected neck or shaft lesion (J)

4 = Definite neck or shaft lesion (K, L, M or N)

5 = Suspected juxta-articular lesion (P)

6 = Definite juxta-articular lesion (Q, R, or S)

7 = Broken articular surface (T, U or W)

8 = Osteoarthritis (X)

9 = Operation (Z)

Although this may appear more complicated than other schemes its design is meant to see at a glance the worst affected men by the allocated number and also to enable easy selection of information for statistical evaluation. For example, by programming the computer to select all men with numbers 7, 8 or 9 on radiographic summary, the number of broken joint surfaces on the register can be rapidly obtained. For a more detailed account of the computer storage system, see Evans (1977).

INCIDENCE OF CAISSON DISEASE OF BONE AND THE NEED FOR FURTHER RESEARCH

Reported incidence figures for caisson disease of bone vary

greatly. As well as depending upon the type of exposure and decompression table used (if any), much depends on the interpretation of the radiographic examination. The Medical Research Council Decompression Sickness Central Registry have a panel of experts who are allowed to report a radiograph as 'suspicious' of a necrotic lesion as well as 'definite'. Table 1 shows details of typical incidence figures for 'definite' lesions. It is not clear in some of the earlier reports in the literature whether or not bone islands were included as a form of caisson disease of bone. The largest samples of men tend to be from contracts involving work in compressed air where many workers may be exposed simultaneously to raised pressure daily for months on end, rather than from diving operations which often involve relatively few men sporadically exposed to pressure. The non-use or non-adherence to decompression schedules leads to a greatly increased percentage of men with definite radiological lesions and this is exemplified by studies of commercial native shell fish divers in Japan (Ohta and Matsunga, 1974; Kawashima, 1976).

Of the 'population at risk' by exposure to excess pressure, many prove to be susceptible to the development of caisson disease of bone, even if they adhere to recommended decompression schedules and even if they never experience any form of acute decompression sickness. The importance of research work into the aetiology, early diagnosis and management of any disease produced solely by the working environment can be criticised on the grounds that the best form of treatment in such circumstances is prevention, and that caisson disease of bone is easily preventable by not exposing men to work in compressed air or on the seabed. However, if

TABLE 1 : PREVALENCE OF CAISSON DISEASE OF BONE

A. COMPRESSED AIR WORKERS

AUTHOR	NUMBER OF MEN EXAMINED	NUMBER WITH LESIONS	% LESIONS	NOTES
BELL et al. (1942)	32	24	75	N.Y. Dept. of Labor, Decompression tables not stated
GOLDING et al. (1960)	83	10	12	1958 tables for D.C. experienced men - all suffered 'bends'
McCALLUM et al. (1966)	241	47	19	1958 tables
M.R.C. DECOMPRESSION SICKNESS PANEL (1971)	171	44	26	Modified 1958 tables
NELLEN AND KINDWALL (1972)	169	59	33	Modified 1922 New York Code
TROWBRIDGE (1977)	2,200	383	17	All the workers with radiographs on M.R.C. Central Registry file

TABLE 1 : PREVALENCE OF CAISSON DISEASE OF BONE

B. DIVERS

AUTHOR	NUMBER OF MEN EXAMINED	NUMBER WITH LESIONS	% LESIONS	NOTES
HERGET (1948)	47	13	36	Foreign literature quoted by Elliott and Harrison (1970)
HERGET (1952)	90	29	32	
SLØRDAHL (1953)	13	3	23	
ALNOR (1963)	131	72	55	
KIRYAKOV (1964)	29	19	65	
ELLIOTT AND HARRISON (1970)	350	14	4	R.N. Clearance divers. R.N. tables but also experimental dives
OHTA AND MATSUNAGA (1974)	301	152	50.5	No decompression schedules used by these divers
OHIWA AND ITOH (1975)	95	3	3.0	Japanese navy divers. Standard decompression schedules
KAWASHIMA (1976)	450	268	59.5	Includes bone islands
THICKETT AND EVANS (1977)	2,516	60	2.4	All divers on M.R.C Central Registry file

businessmen, economists, civil servants, politicians and other people not themselves at risk feel that such work is necessary to provide better communications and sources of energy, then such work will continue. The 'challenge' of the discovery of sub-sea oil and natural gas deposits beneath large areas of continental shelf has in recent years meant a great increase in the numbers of commercial divers now at risk. These are otherwise fit, highly trained young men in whom the structural failure of a shoulder or a hip joint could mean the end of a career.

Medical science must therefore do as much as possible not only to keep insisting on the adherence to the best decompression schedules available but also to ensure that no chance of improving the safety of the tables is overlooked. It must also pursue investigations into the disease process of caisson disease of bone, in order to determine why the condition occurs and what is the best method of management. The following chapters will outline the present difficulties, as I see them, of research into the aetiology and early diagnosis of this disease.

CHAPTER 2 - THE PROBLEM OF AETIOLOGY

THEORETICAL ASPECTS

Only when a patient with known caisson disease of bone dies or has surgical treatment for his condition is there the possibility of obtaining material from a lesion and its bony surroundings for laboratory investigations. The histopathology discussed in Chapter 1 was based on reports of specimens obtained in this way and these specimens are all of longstanding osteonecrotic lesions.

Kahlstrom et al. (1939) described the histology (a) of a biopsy specimen obtained from an advanced hip lesion and (b) of a specimen from a patient who died aged 61 from a bronchogenic carcinoma. Other reports of single cases all reported late findings (Walker, 1940; Swain, 1942; Seze et al. 1951; Laufer, 1957). McCallum et al. (1966) gave a detailed report of histological examination of the humeral head and femoral head lesions of a compressed air worker who had died during treatment of decompression sickness, and Weatherley et al. (1977b) gave a further instance of a man with juxta-articular lesions (without structural collapse) and a shaft lesion. The pathology of these lesions has already been discussed and the strong evidence of repair noted. This repair by appositional new bone formation was previously called 'creeping substitution', a term first used by Phemister (1920) in talking about remodelling of bone transplants, but is probably best avoided when referring to areas of repair of osteonecrosis as the dead bone is not initially absorbed (Bobechko and Harris, 1960) and even though it may be slowly absorbed during the following months or years, unresorbed dead bone fragments are found within the new trabeculae even after several years (Catto , 1965).

Thus, historically, the lesions of caisson disease of bone were regarded as being similar pathologically to other forms of osteonecrosis, and to be 'ischaemic' or 'avascular' and to undergo repair by means of 'revascularisation'. If the lesions are regarded as being ischaemic then the question must be asked as to how the normal blood supply is affected. This could be due to arterial occlusion (intraluminal or by external pressure), arterial spasm or other intramural changes, or even capillary bed or venous occlusion preventing further arterial inflow. Agents postulated in this respect will be discussed later, but first the available evidence for the time-course over which they would need to act will be given.

A time of six hours longer appears to be necessary before the interruption of blood supply gives rise to osteocyte death, and therefore permanently damaged bone. The evidence for this comes from the experimental interruption of the blood supply to the head of the femur in dogs by subcapital ostectomy plus rotation of the free head for various set times to occlude the only vessels remaining to the head, those in the ligamentum teres. One week later, the viability of the bone was tested by examining its uptake of tetracycline, using the ultraviolet fluorescence test (Woodhouse, 1962). Similar experimental work in rabbits by Rosingh and James (1969) demonstrated loss of DNA from osteocyte nuclei after 6 - 12 hours, this test again indicating permanently damaged bone.

Indirect evidence for a similar time interval being important comes from observing the uptake of radioisotope labelled aminoacids by osteocytes at successive time intervals after the removal of normal bones from sacrificed laboratory animals. This experiment was performed by Kenzora (1972). He found that only half as many osteocytes were labelled twelve hours after removal of the bone as

compared with the number labelled at zero time.

Of the postulated agents which might cause obstruction of bone blood flow for 6 hours or more, the one which springs to mind most readily in caisson disease of bone is gas bubbles, which are commonly believed to be the underlying cause of acute decompression sickness. Unfortunately, this is most awkward from a theoretical point of view, because it immediately raises the question 'How long can a gas bubble exist in a given environment?' and there really seems to be no good theoretical answer at present. From a medical point of view, however, the answer must be that gas bubbles can exist in the body for more than six hours because an attack of 'bends' pain (normally responding to recompression and therefore presumably due to a bubble) when not treated by recompression can last many hours. This still does not mean that bone damage is directly attributable to gas bubbles. The presence of gas bubbles arising during decompression could lead to a sequence of changes including occlusion of vessels by other substances in the circulation (such as platelet clumps, or fat globules) adhering to the blood/bubble interface.

GAS BUBBLES

It has been shown that showers of gas bubbles can be detected by Doppler ultrasonic transducer recording over the heart and great vessels during decompression from excess pressure even in the absence of clinical symptoms of decompression sickness (Spencer and Clarke, 1972; Evans et al., 1972). Also, analysis of intravascular gas bubbles liberated by rapid decompression has shown them to be largely nitrogen (Bert, 1878). Theoretically, such bubbles could be formed within the circulation during decompression either 'de novo' from plasma supersaturated with nitrogen or by the expansion

of pre-existing minute gas nuclei already present in the circulation. The work of Harvey et al. (1944a, 1944b) discussed these possibilities and stated that in a homogeneous liquid at rest containing dissolved gas the drop in pressure necessary for bubble formation 'de novo' would be greater than 100 atmospheres. As this does not accord with the experience of bubble formation in animals and man, where intravascular bubbles occur with a much smaller decrease in pressure, they concluded that pre-existing gas nuclei or bubbles must be present. They could not find gas nuclei in the blood and so it was felt that perhaps bubbles were attached to hydrophobic cracks in vessel walls. However, with present knowledge of biological membranes, supplemented by electron microscopy, there is no evidence of any hydrophobic cracks, so the mode of formation of the bubbles remains hypothetical. A novel explanation for the spontaneous appearance of bubbles in the body has recently been suggested by Walder and Evans (1974), who postulated that the spontaneous fission of the Uranium 238 normally present in the body releases sufficient energy to generate a bubble where one was not present before.

From pressure considerations, bubbles should form more readily in veins than arteries (Harvey et al., 1944a). This accords with findings in fatal rapid decompressions of laboratory animals (see, for example, Gersh et al. 1944; Antopol et al. 1964). While such experiments produce widespread gas bubbles intra and extravascularly these show no tendency to localise in bone (Gersh, 1945), whereas in post-mortem material from divers and compressed air workers, ischaemic lesions in the soft tissues have not been reported, although necrotic lesions are seen in the bones.

Whether bubbles are carried to intra-osseous vessels by the

circulation or whether they arise within the bones intra- or extra-vascularly is unknown. Attempts to simulate the bone lesions by intra-arterial injection of particulate matter (Kistler, 1934; Cox, 1973) assume the former, but the latter must also be considered. The bone marrow of the shafts of the long bones in adult humans is mostly yellow (fatty) but there are usually patches of red (haemopoietic) marrow in the proximal end of the femora and humeri (Piney, 1922). Nitrogen is five times more soluble in fat than in water and plasma (Vernon, 1907) and the tissues containing large proportions of fat are yellow and the adipose tissue situated subcutaneously and around viscera. That marrow fat dissolves comparable volumes of nitrogen to the fats studied by Vernon was confirmed by Campbell and Hill (1931). In addition to saying that this local concentration of fatty tissue within long bones might mean more dissolved nitrogen would be available to form bubbles during decompression from excess pressure than elsewhere in the body, local circulatory differences might be very important. The detailed blood supply of long bones remains controversial, though the detailed studies of Brookes (1971) demonstrate a supply from within progressively outwards. This would mean that intravascular bubbles arising within the bone marrow would have to traverse the capillary bed of the cortex before being free to pass onwards in the venous system to be lost by diffusion from the pulmonary capillary bed. Other factors which might act to influence bone circulation during decompression are an increase in intra-medullary pressure greater than the simultaneous increase in femoral artery pressure (Harrelson and Hills, 1970), causing local tissue water displacement and a decrease in bone blood flow (Hills and Straley, 1972). However,

both these changes only last 5 to 15 minutes and it is difficult to see how they could be of importance in causing significant vascular obstruction. On the other hand, a prolonged increase in intramedullary pressure by saline infusion in experimental animals has been claimed to produce diaphyseal necrosis (Larsen, 1938). In man, measurement of intramedullary pressure does not show any increase in areas of aseptic necrosis (though there may be loss of the transmitted arterial pulsation) (Miles, 1955, 1959) or any increase if five millilitres of fluid is injected (Arlet, 1971). The only circumstances in man in which intramedullary pressure seems to be markedly raised is acute leukaemia (Petrakis, 1954) and osteonecrosis is not a recognised complication.

That both intravascular and extravascular bubbles occur in many tissues following rapid decompression of experimental animals has been claimed from microscopic examination of many preparations following death or sacrifice as soon as possible after decompression. To prepare the tissues for microscopy rapid freezing and dehydration has been used (see, for example, Gersh, 1945) and many sources of artefect come to mind when reading the papers in which such work is reported. Not the least of these is that air must be introduced into the tissues by the knife blade as the dry metal surface of the blade cannot be made free of gas mincromuclei (Harvey et al., 1944b) In addition, when water freezes it expands and the tissue might be distorted by ice crystals removed during subsequent fixation leaving a 'hole' appearing to represent a gas bubble. However, gas bubbles have been observed during decompression in living animals (Bert, 1878; Lever et al., 1966; Heimbecker et al., 1968). Although both intravascular and extravascular bubbles were seen in subcutaneous tissue, there was no obvious relationship or communication between them. Lever et al. (1966) could not determine the source

of intravascular bubbles but found that they occurred in suboutaneous, mesenteric and femoral arteries before they appeared in the corresponding veins. Wagner (1945) recorded this same finding in cat pial arteries and veins. On the other hand, Heimbecker et al. (1968) reported that bubbles first appeared in the capillaries and venules.

Thus, there seems to be plenty of evidence that gas bubbles are present during decompression, but how and where they arise remains a mystery.

Once a gas bubble is present in the arterial blood supply of bone it becomes easier to reason how it could remain there for sufficient time to produce tissue damage, than it is to speculate how it originates. If the bubble size is sufficient to occlude blood flow the surrounding tissue remains supersaturated with nitrogen as decompression proceeds. The bubble will maintain its size because there are no surrounding tissues less saturated with nitrogen into which gas from the bubble can pass by diffusion. The bubble will persist until the partial pressure of nitrogen in the bone marrow falls. It is impossible to say how long this would take, and even if a decompression only lasted one or two hours, bubbles might become trapped in the circulation in this way for the time necessary to cause bone and marrow cell death. The circumstantial evidence for the ability of bubbles in other sites after decompression to remain this long has already been mentioned.

There seems to be no reason why extravascular bubbles might not cause occlusion of small blood vessels as they expand during decrompression. This might be especially so in areas of the body which can be regarded as semi-closed rigid compartment, as for example the marrow cavities of the long bone (Rasgon et al., 1951).

The same sequence of reasoning for the persistence of these bubbles could then be used as for intravascular bubble.

OTHER WAYS IN WHICH GAS BUBBLES MIGHT CAUSE CIRCULATORY OCCLUSION

Much work has been performed to look for ways in which intra-arterial bubbles could cause plugging of vessels by stimulating the production of platelet thrombi, causing fibrin deposition or lipo-protein aggregation, or anything else that might act as an embolus. This subject has recently been reviewed by Elliott et al. (1974).

There is a marked circulating plasma deficit following rapid decompression (Cockett et al., 1965) and the associated increase in haematocrit and blood viscosity (Guest et al., 1974) make any observed changes in specific coagulation factors (summarised by Guest et al., 1974) difficult to interpret. This haemoconcentration may explain the marked alterations in the haemodynamics of the capillary bed observed in the Hamster cheek pouch during decompression by Heimbecker et al. (1968).

Warren et al. (1973) report finding platelet aggregates and thrombi, lipid droplets and endothelial 'damage' in relation to blood/bubble interfaces. I feel that observations made using the electron miscroscope have to be interpreted with caution as gas bubbles are not a normal feature in living cells and body fluids (Harvey et al., 1944a), and the observer has no'normal' for comparison. The bubble itself presents merely as a 'hole' and such spaces might be produced by artefacts in these thin sections. However, evidence of platelet involvement is supported by the findings of thrombocyto-paenia and decreased platelet survival times which have been reported after rapid decompression of miniature swine (Stegall et al., 1973). This same study found no change in serum fibrinogen levels.

These changes have been recorded in relation to explosive decompressions of laboratory animals which are often fatal and would certainly be fatal in man. No such changes have been discovered following standard decompressions of human beings. The main human study is that of Philp et al. (1972). This gives haematological and biochemical data collected before and after 16 dives, 12 of which produced symptoms related to decompression (3 divers required therapeutic recompression). Marginal increases in haemoglobin concentration and haematocrit, all well within the range of normal values and not reaching statistical significance, were recorded, together with a more marked fall in platelet count. Detailed coagulation studies gave some abnormal values which just bordered on statistical significance. The fall in platelet count was confirmed by Martin (1973). Plasma and urine chemistry showed no significant changes. Serum lipids were measured pre- and post-dive by Cockett et al. (1976) but no significant change was seen in cholesterol or phospholipid levels, and a rise was found in mean unesterified fatty acids. Triglyceride levels were not measured in this study.

All this may be summarised by saying that the changes seen when normal human decompression schedules known to produce bone necrosis are used are insignificant and remain within the range of normal values in all cases, so that there are not good grounds at present for believing any haematological or biochemical factors to be involved in the clinical situations producing caisson disease of bone.

EPIDEMIOLOGICAL DATA

Clues to factors important in the aetiology of a condition may be obtained from epidemiological studies as well as from theoretical

and experimental work. This is especially so when the presence or absence of a suspected causal agent cannot be measured directly. It is not possible during any one decompression to know if gas bubbles are present intra- or extra-vascularly within the long bones as they do not produce symptoms. Other forms of decompression sickness attributed to gas bubbles do produce symptoms and therefore if the aetiology of caisson disease of bone is similar a correlation with other forms of decompression sickness might be expected. This has been investigated by comparing the 'bends rate' (the incidence of type 1 decompression sickness requiring recompression) with the rate of later developing osteonecrosis (Walder, 1970). A correlation was found but many men with bone lesions denied a history of acute decompression sickness. This may be because supersaturation in bone marrow may produce damage at gas-tension levels below those causing symptoms of decompression sickness elsewhere in the body (Harvey, 1974). Walder also found that the men with bone lesions tended to have worked at higher pressures and undergone more decompressions, but it is not possible to deduce anything about aetiology from this. It would be expected both if the lesion was due to a cumulative effect of repeated decompressions or to a single ischaemia-producing episode (as the greater number of decompressions, the greater the chance of such an episode occurring).

Studies have also been made of the site of bone lesions (which appear to be generally restricted to the humeral head, neck, and upper shaft, femoral head, neck and upper shaft, lower femoral shaft, and upper tibial shaft); association of lesions with fatness as measured by skinfold thickness; and association of site of femoral lesions with leg length (Decompression Sickness Panel of Medical Research Council, 1971) but no significant

associations were found.

The incidence of lesions might vary with different decompression schedules used on separate compressed air work contracts, but this is difficult to determine as many workers move from one contract to another. Despite this, a report of experience with the most conservative tables in widespread use, the Washington State tables first used in 1964, found no clinical or radiological evidence of bone necrosis (Behnke and Jones, 1974). This is perhaps not surprising as the work at pressures greater than 17 p.s.i.g. only lasted 47 days and the shift length was limited at the higher pressures. Furthermore, only 16% of the workers were available for follow-up at one year and even less in following years.

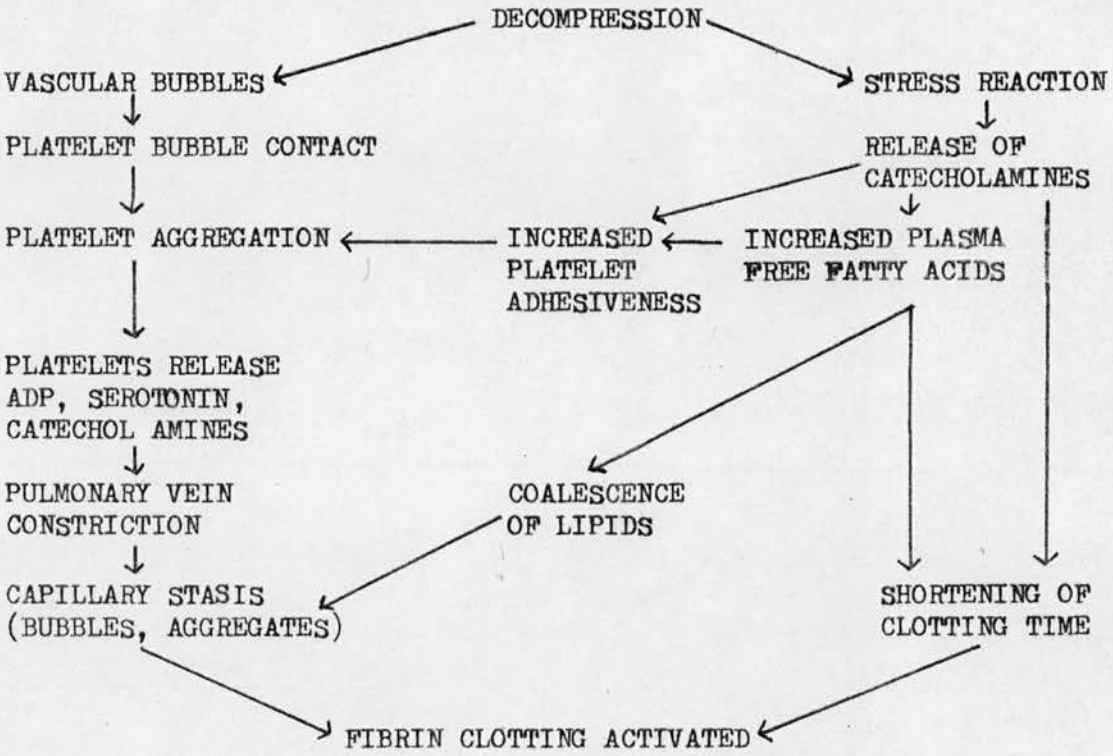
SUMMARY

Chinese Syntax

The one overriding observation implication bubbles alone, unaccompanied by any form of fluid or solid vascular obstruction, in the pathogenesis of acute decompression sickness is the dramatic response of symptoms to recompression. This would not be expected if major circulatory or coagulation changes were important. The same cannot definitely be stated for bone necrosis, which is only discovered months or years later. However, it seems most logical at present to assume bubbles have an important role and to say that any of the chain of events postulated in figure 1 remain entirely speculative. Other suggestions, such as that made by Harrelson and Hills (1970) that the bone lesions might be a result of rapid compression rather than decompression do not accord with clinical observations, such as the occurrence after slow compression and single rapid decompression in submarine escape (James, 1945) and the very high incidence in Japanese divers not using any decompression schedule (Ohta and Matsunaga, 1974). Similarly, to argue that the

FIGURE 1

a. SIMPLIFIED SCHEME OF POSSIBLE INVOLVEMENT OF PLATELETS, LIPIDS AND FIBRIN IN DECOMPRESSION SICKNESS SYNDROME (PHILP et al., 1971)



b) SCHEME PROPOSED BY STEGALL AND SMITH (1976)

i. DECOMPRESSION RESULTS IN TISSUE SUPERSATURATION

ii. SUPERSATURATION PLUS OTHER FACTORS LEAD TO INTRA- AND EXTRAVASCULAR BUBBLE FORMATION.

iii. BUBBLES ARE FOREIGN SURFACES TO THE BODY AND MAY DAMAGE TISSUES.

iv. DAMAGED TISSUE AND THE PRESENCE OF FOREIGN SURFACES MAY CAUSE:-

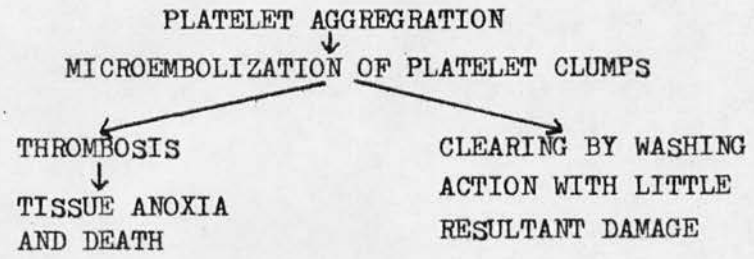
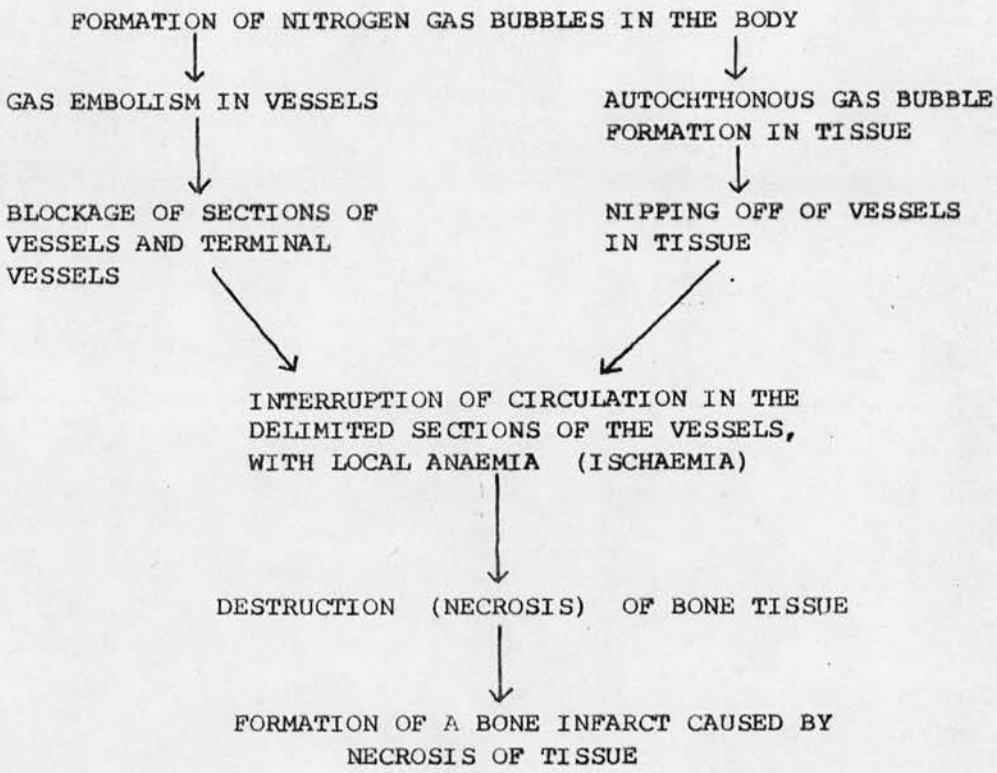


FIGURE 2

BONE DAMAGE AFTER DECOMPRESSION FROM EXCESS PRESSURE

(CAUSAL PATHOGENESIS) (WUNSCHE and SCHEELE, 1973a)



bubbles seen in the hamster cheek pouch experiment are secondary to the altered haemodynamics with agglutination of red cells exposing hypothetical hydrophobic regions to allow formation of gas nuclei (End, 1971) seems unsound, as the gas nuclei should theoretically be present all the time rather than formed de novo (this was the reason for research to try and find evidence of hydrophobic cracks), and illogical, as no significant haemodynamic changes have been observed in man. After all, it is men and not animals that develop caisson disease of bone.

Figure 2, given for comparison, is translated from the article of Wünsche and Scheele (1973a) and restricts itself to possible mechanisms of pathogenesis by bubbles alone.

It is difficult to devise ways of investigating possible steps between the presence of a bubble and the development of an area of bone necrosis. This is because even if we were certain of what to look for, and where to look for it, the question of when to search during a series of decompressions using human schedules known to give rise to caisson disease of bone would remain, as the bone lesions arise asymptomatically.

CHAPTER 3 - THE PROBLEM OF EARLY DIAGNOSIS

HISTORICAL INTRODUCTION

Caisson disease of bone was not reported until many years after the acute forms of decompression sickness. The first reviews of workers with long term complaints of stiff painful joints were those of Bornstein and Plate (1911) and Bassoe (1913). Both papers found 'arthritis deformas' of affected hip and shoulder joints on radiological examination. Bassoe appears to have seen a shaft lesion in the upper tibia and says that radiological opinion was that the observed appearance was of bone reconstruction following an atrophic process. Erdman (1913), in reviewing the pathology of twenty fatalities related to compressed air work on the East River tunnels from New York to Long Island, states that in one case necrosis of the femur was found, apparently secondary to thrombosis of the medullary artery, but gives no more details which might indicate the typical shaft lesion.

It is desirable to detect bone damage before death or the development of crippling arthritis, and this means detecting lesions before symptoms develop. If radiography is performed only when joint symptoms have started, the lesions found will be irregularity of the joint surface, and such lesions are irreparable no matter how long nature is allowed to try and effect a repair. The irregularity may be due to collapse of weight bearing areas into the underlying cancellous bone or of sequestration of a fragment of cortex; alternatively the appearance may be of a more generalised degenerative arthritis. If radiography is performed on a survey basis, before the development of symptoms, then earlier stages in the development of bone lesions might be found, and this has proved

correct. The classification of these lesions has already been given in Chapter 1.

An ideal might be to perform radiography before a man starts to work in compressed air, both to have a baseline to compare with any doubtful changes seen on radiological examination after a period of exposure and also to detect any pre-existing lesions for which later insurance claims for workmen's compensation could be resisted. This second point was one of the recommendations of Bornstein and Plate in 1911 but it is still not a legal requirement for compressed air workers in Great Britain.

Despite the many reports of caisson disease of bone since 1911, the main emphasis of medical attention to decompression sickness was on acute manifestations until comparatively recently. It was only then that the increasing stringency of decompression tables and legal insistence on workers adhering to them had led to an acceptable incidence of acute decompression symptoms (of the order of 1-2% of decompressions). Radiological surveys as listed in Table 1 showed that these workers still had a significant prevalence of bone lesions. The earliest a definite bone lesion has appeared on these surveys is three and a half months after starting work in compressed air (Walder, 1976). As emphasised in the note on pathology, radiographic changes at earliest show a repair reaction and do not demonstrate dead, as compared to living, bone. However, it is desirable to diagnose the bone damage as early as possible so that a worker may be advised that he is known to be susceptible to osteonecrosis. He could also be advised against further decompressions, or to restrict load-bearing activity on the affected joint in juxta-articular lesions of the humeral or femoral head.

POSSIBLE MEANS OF EARLY DIAGNOSIS

A. DETECTION OF GAS BUBBLES

Assuming intravascular gas bubbles to be of major aetiological importance, the earliest possible diagnosis of any form of decompression sickness would be the detection of such bubbles. This has been done by praecordial monitoring by Doppler ultrasound (Spencer and Clarke, 1972; Evans et al.,1972). Showers of bubbles were recorded even during symptom-free decompressions. This may explain why bone necrosis has been found clinically in compressed air workers and divers who have never experienced symptomatic acute decompression sickness. This assumes a hypothesis of the form :

> 'Acute forms of decompression sickness are caused by gas bubbles, as they respond to recompression. Caisson disease of bone is caused in the same way but is not manifest at the time because for some reason not at present understood the gas bubbles do not lead to the development of painful stimuli within the bone.'

However, it is possible to state virtually the opposite, for example :

> 'Even if bubbles cause acute forms of decompression sickness, they do not cause bone lesions as they would not remain for the length of time necessary to cause bone death. Caisson disease of bone need not be due to any single episode of decompression but to some other change or series of changes taking weeks or months to develop.'

Although neither of these statements can at present be definitely negated, the reasoning against the latter has already been given in the discussion of aetiology. I cannot think of any experiment that

would help to decide for or against either of these statements, even using our ability to detect bubbles. If people with no previous hyperbaric exposure performed repeated bubble-free decompressions (as detected by praecordial ultrasonic monitoring) and bone necrosis still occurred, a valid objection would still be that this only implies an absence of intravascular bubbles. Extravascular bubbles could still be present and important. It should be noted that considerable lengthening of present decompression schedules would be required to produce a bubble-free decompression.

Doppler ultrasound is not a painful, harmful or invasive technique, and could be very useful. While it may not be practicable to monitor a large group of tunnel workers undergoing decompression every day, a group of three or four divers having a decompression lasting several days after saturation diving would require much less equipment and monitoring might prove very useful in avoiding acute decompression sickness and recompression at very great pressures.

B. BIOCHEMICAL CHANGES

An alternative approach to possible early diagnosis would be the detection of some biochemical change caused by bone ischaemia. However, it would seem unlikely that any substance is going to pass into the circulation in measurable amounts while decompression is in progress, both because of the local circulatory shutdown and also because tissue death may be necessary before measurable amounts of substances leak into the circulation. Considering an ischaemic lesion of a long bone, substances might arise from :

1.	Bone cells -	enzymes	e.g. lysosomal
11.	Bone matrix -	collagen	e.g. hydroxyproline
111.	Bone Mineral -	hydroxyapatite	e.g. calcium, phosphate
1V.	Bone Marrow -	haemopoietic	e.g. ferritin
	-	fatty	e.g. cholesterol, triglycerides

Attempts to measure any of these substances have been rather haphazard. There are several reports of measurements before and after rapid decompression of laboratory animals but few in man. The major stresses, including air embolism and death, in the experiments on animals, mean that changes in surviving animals after the same rapid decompression might be caused by the general metabolic upheaval associated with almost dying rather than discreet changes in any particular tissue. The studies in man seem more relevant, though by studying a single dive it would not be valid to relate any positive findings to bone damage. Negative results would be helpful. Philp et al. (1972) studied a group of sixteen divers, twelve of whom had symptoms of minor forms of decompression sickness. They found no statistically significant differences following decompression in serum cholesterol, triglycerides, calcium, inorganic phosphate or osmolality. Urinary calcium and inorganic phosphate were measured but expressed per unit creatinine excretion. No change was found except for an increase in urinary calcium in the group of four divers without symptoms. This finding was not confirmed by Heyder and Tappan (1973b) who observed a decrease (not statistically significant) of total urinary calcium on the first day after a forty five minute exposure to 2 ATA. After a similar exposure to 7 ATA they observed a significant decrease in total urinary inorganic phosphorus and an increase in urinary hydroxyproline excretion.

The only work which has claimed to produce bone necrosis in man or animals following hyperbaric exposures and has measured biochemical parameters in the same subjects appears to be the work of Stegall and Smith in miniature swine. (Smith and Stegall, 1974 ; Stegall and Smith, 1976). The hyperbaric exposure used was to 60 feet of sea water for six hours and decompression was rapid (30 feet per minute). The most measurements made were of haematological data, but they also recorded that serum calcium, uric acid, alkaline phosphatase, and cholesterol showed no significant change. Serum values of the enzymes glutamo-oxalate transaminase (GOT), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) showed a rise above baseline levels, but did not exceed the reported normal range of values for miniature swine. It is not clear in the published tables (Stegall and Smith, 1976) whether the figures were for single dives or are averages of multiple dives. They interpreted these changes as a reaction to the stress of rapid decompression. No measures of serum ferritin or total urinary hydroxyproline excretion have been made on this group of animals.

Other experimental work has involved measurements on artificially-induced bone necrosis. The animal model used was developed by the Medical Research Council decompression sickness team in Newcastle-upon-Tyne and is reported by Cox (1973, 1974). Glass microspheres of 45 - 120 μ m diameter were injected into one iliac artery, and histological examination of the ipsilateral femur showed the development of areas of bone and marrow necrosis. This work in adult rabbits was repeated in adolescent rabbits (ten weeks old) by Spivey and Park (1973, 1974) with the femoral artery temporarily occluded in an attempt to produce ischaemia of the hip and proximal

femoral area. They successfully produced lesions which radiologically showed a striking resemblance to Legg-Perthes' disease and histologically showed bone death. This model has now been used to measure a number of parameters. Extrapolating the results obtained to caisson disease of bone assumes that intravascular occlusion by bubbles or particulate matter is of aetiological importance.

C. URINARY HYDROXYPROLINE EXCRETION

The amino acid hydroxyproline occurs as a component of collagen (about 13%), elastin (1 - 2%), and the Cl_q subcomponent of complement (Reid, 1974). The twenty four hour urinary excretion of total hydroxyproline (free amino-acid plus that present in urinary peptides) is increased when collagen is being broken down (e.g. when bone is being replaced by tumour or matrix is being lost in rickets or osteomalacia). A full list of conditions in which excretion is increased is given by Laitinen et al. (1966). It should be noted that urinary excretion is only a small proportion of hydroxyproline production within the body. Wiegmann et al. (1976) gave a figure for urinary excretion of 10% of the total hydroxyproline formed by catabolism in the body.

Deiss (1974) suggested that if some of the necrotic bone in an area of osteonecrosis was resorbed then this might be detected by increased urinary hydroxyproline excretion. If 10% of the body weight is the skeleton, and 19% of this is collagen (Eastoe and Eastoe, 1954) then collagen in bone is almost 2% of total body weight (Stevens, 1963). This is an appreciable proportion but with osteonecrosis only a small part of the skeleton would be involved.

Following the Medical Research Council rabbit model operation using intra-arterial glass microspheres, Weatherley et al. (1977 measured serial 24 hour total urinary hydroxyproline excretion and

showed a significant increase in the animals which developed osteonecrosis. This started five days after operation and continued for the twenty one days measurements were made. Later unpublished data showed a return to baseline levels by 42 days after operation.

In this work the increases had to be expressed in terms of the baseline excretion of the same rabbit as there is a large range of individual variation of normal excretion. This is also found in man. Excretion in man is also increased in children (related to growth as in four hypopituitary dwarfs the daily excretion rose after administration of human growth hormone (British Medical Journal 1963)) and with dietary intake of foods rich in gelatin (Ziff et al., 1956; Prockop et al., 1962). There is a diurnal rhythm of excretion with greater excretion overnight (Mautalen, 1970) so samples from 24 hour collections have to be measured.

The only report of an increase following hyperbaric exposure in human subjects was an experimental exposure of twenty divers in the U.S. Naval Submarine Medical Research Laboratory using an exposure and decompression profile designed to be safe and very unlikely to produce bone necrosis (Heyder and Tappan, 1973b). The same authors have been unable to reproduce this result in severe decompression (resulting in one third fatality) of Sprague-Dawley rats (Heyder and Tappan, 1973a) and further human experiments (Heyder and Tappan, 1974). The human subjects had some dietary restriction (no ice-cream, gelatin desserts, or soft candy) but were not subjected to a hydroxyproline-free diet. Preliminary studies of hydroxyproline excretion during saturation dives on naval and commercial divers have shown day to day variations with peak values on the same day for all the men on the dive (Weatherley, unpublished data), strongly

suggesting that variation in diet is important.

D. SERUM ALKALINE PHOSPHATASE

Alkaline phosphatase is an enzyme present in most cells and in serum. It has a wide specificity and catalyses the hydrolysis of a variety of phosphoric acid monoesters. In many bone diseases the amount in the serum is greatly increased, as it also is in subjects with biliary obstruction. Histochemical localisation shows intense staining of osteoblasts and osteocytes with no activity demonstrated in osteoclasts, whereas the reverse is true of acid phosphatase, which is present in large amounts in osteoclasts and foreign body giant cells (Burstone, 1960). Despite this, serum levels are raised in disease states associated with active bone reorption.

Measurement of serum alkaline phosphatase showed no change after hyperbaric exposure of miniature swine (Stegall and Smith, 1976) or after operation to artifically induce bone necrosis by intra-arterial glass microspheres (Gregg and Walder, 1977).

E. SERUM FERRITIN

Because the areas of bone necrosis in divers and compressed air workers have associated marrow death, Gregg (1977) proposed measurements of serum ferritin levels as possibly useful in osteonecrosis. Measurement of ferritin in various tissues had shown it to be present particularly in the liver, spleen, and bone marrow (Powell et al., 1975) and raised serum levels had been reported following damage to liver cells (Prieto et al., 1975 ; Eastham et al., 1976).

Few measurements have been reported following hyperbaric exposure (Admiralty Marine Technology Establishment, 1978). Using the Medical Research Council rabbit model and artificially induced bone

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and marrow necrosis, Gregg et al. (1977b) reported a significant increase in serum ferritin levels 24 hours after operation. This is a potentially useful and sensitive technique to apply to the study of early dysbaric osteonecrosis as the diurnal and daily fluctuation is only ± 12% of the mean value in man (Siimes et al., 1974) and ± 19% of the mean value in a control group of rabbits following sham operations (Gregg et al., 1977b).

The only other situation in which marrow necrosis occurs in man is in sickle cell disease. Serum ferritin levels are raised in these subjects (Peterson et al., 1975). It therefore seems possible that elevated levels might be able to detect marrow necrosis following hyperbaric exposure at an early stage.

F. COLLAGENOLYTIC ENZYMES

Techniques are now available which are claimed to give an estimation of collagen breakdown without measurement of hydroxyproline excretion (as mentioned in C. above). As experiments have shown increased hydroxyproline excretion in bone necrosis these merit consideration.

Collagen breakdown within the body appears to be initiated by the collagenase enzymes. These are specific enzymes which can attack the triple helix of native collagen and produce a cleavage across all three polypeptide chains at a point three quarters of the way from the N-terminal of the collagen molecule. (Harris and Krane, 1974). A number of collagenases have been found in the culture media of mammalian tissues including bone (Shimizu et al., 1969). Collagenolytic enzyme activity has also been detected directly from extracts of animal skin and human synovial membrane (Nagai and Hovi, 1972). Lysosomal preparations have

also shown activity against native collagen and this appears to be caused by the carboxyl proteinase Cathepsin B, found in human livers (Burleigh et al., 1974). Any possible importance of this in vivo has yet to be established.

The peptides formed by breakdown of collagen by collagenase are further degraded by aminopeptidases. Many aminopeptidase enzymes have been isolated but most are unable to attack the proline and hydroxyproline amino-acid sequences of collagen. Aminopeptidase activity in bone cells appears to have been first reported by Lipp (1959) who found it in osteoclasts and some osteocytes but not in osteoblasts. A more detailed review is reported by Burstone (1960). A profile of activity of these enzymes in rat cartilage against various amino acid terminal groupings is presented by Hirschman and Hirschman (1971) and this shows very little activity for proline groupings. Unfortunately, I cannot find similar data for bone.

In 1970 a specific proline iminopeptidase (PIP) was measured in human serum which split the amino-acid sequence - Gly - Pro - Leu - Gly - Pro - X (as present in the apolar regions of the collagen molecule) between the leucine and glycine residues (Gries et al., 1970). This has been shown to be significantly increased in patients with Paget's disease. Although there remained an overlap with the normal range the serum PIP could be correlated with both plasma alkaline phosphatase activity and urinary 24 hour total hydroxyproline excretion. (Whiteley et al., 1976). This same paper found no significant difference in serum PIP values of a group of patients with liver disease from those of the normal population and found that values were not affected by the addition of gelatin to the diet.

Whether elevated values of serum PIP would be found in early stages of bone necrosis is problematical. It might depend on the source

of the enzyme. If it was found locally in bone cells either to be secreted or to diffuse into the surrounding extracellular fluid to initiate breakdown of neighbouring collagen then this would require viable bone cells. When subjected to death by removal of bone from the body 50% of osteocytes failed to incorporate tritiated proline and tritiated cytidine as shown by autoradiography after twelve hours and 92% after 24 hours (Kenzora, 1972: data for adult rabbit cortical and cancellous bone). Therefore, in circumstances causing a region of bone and marrow death the bone cells in that region might also be expected to die rapidly and very little induction of collagenolytic enzymes would occur locally within the area of necrosis. There is a difference here to serum ferritin, the ferritin being present in large amounts in the normal marrow cells and being released when they are damaged, whereas the enzyme PIP might first have to be induced within a living cell. That it may be present in normal bone, however, is indicated by the work of Burstone (1960). He showed microscopic localisation of aminopeptidase by histochemical techniques and found an intense reaction in human osteoclasts plus some staining of periosteum, perichondrium and chondrocytes. This was, unfortunately a general reaction for aminopeptidases and not specifically for PIP.

Despite this reservation concerning cell death (in addition to the more general one of needing a test sensitive enough to detect damage of only a small part of the whole skeleton), if such an assay were to show significant changes it would be more readily useable in man than hydroxyproline excretion measurements, which require 24 hour urine collections and are affected by dietary gelatin intake. There are not yet any reports of serum PIP levels in relation to

artifically induced bone necrosis or decompression-induced bone lesions. The only report of levels during and following human hyperbaric exposure are inconclusive (Admiralty Marine Technology Establishment, 1978).

EARLIER METHODS OF DETECTING THE ESTABLISHED LESION AND REPAIR PROCESSES

Dead bone has the same architectural structure of the mineral framework and the same radiological density as when it was living. (Bobechko and Harris, 1960). The radiograph of a man with a bone containing an area of osteonecrosis will remain normal, with no line of demarcation between living and dead bone, unless:

a. The dead bone undergoes collapse so that the same amount of bone mineral occupies a smaller volume. The strength of bone is proportional to the mineral content (Stevens, 1963) so it is theoretically unlikely to collapse even when the bone is dead. However, repair processes disturb the normal architecture and collapse does occur at the junction of dead and living bone in repairing osteonecrotic lesions.

b. The surrounding bone demineralises because of disuse (either voluntarily to avoid pain, or compulsorily on medical advice or treatment). This is seen, for example, after fractures where the fracture has caused an avascular fragment. With long term immobilisation this fragment maintains the radiological density it had at the time of the fracture, while the living fragments demineralise.

c. A repair process starts and appositional new bone is laid down on dead trabeculae again leading to an absolute increase in bone mineral per unit volume.

The last of these three seems to account for the early radio-

logical changes seen in caisson disease of bone (Davidson, 1976b) although the first also occurs. The second is not relevant as there is no immobilisation or disuse. A percentage increase in bone mineral content per unit volume of 30% may be required to delineate an area from normal surrounding bone (for visual, not densitometric, examination of x-rays). This figure is based on the work of Borak (1942), who produced artificial defects in dried vertebral bodies and showed that there was no change in radiological density unless the lesion removed more than one third of the bone in the direction of the x-ray beam. He did this to explain why osteolytic metastatic tumour deposits did not always show up on x-rays. Osteosclerotic lesions can similarly be missed by radiology, presumably for the same reason (insufficient density change). This agrees with the observation of Catto (1965) that the histological area of bone repair with appositional new bone formation is often greater than the area of increased density seen radiologically. This is also true in caisson disease of bone (McCallum et al., 1966).

A measurement of the rate of calcification might detect new bone formation before an absolute increase in the amount of bone present was evident. This is because once hydroxyapatite is formed and the bone is no longer growing or rapidly remodelling, mineral turnover is at a low level. The turnover ('long term exchange') may result in a steady increase of mineral ('accretion') or not ('recrystallisation'), but experiments using radioactive isotopes of calcium (^{45}Ca) and phosphorus (^{32}P) showed a bone uptake of these labels at a rate showing that in a year only 5 - 20% of the bone calcium was exchanged. (Marshall et al., 1959). Following the administration of radioactive calcium or phosphorus, the baseline of its incorporation into mature bone would therefore be at

such a low level that localised repairing or remodelling bone would be distinguished by a localised increased concentration of radioactivity. Thus formation of bone might be more easily detectable working from a baseline of the slow turnover rate of calcium and phosphate in normal bone and observing a localised increase than by trying to measure the absolute increase of mineral present from an existing baseline of 100% mineralisation. It is far easier to distinguish 2% from 1% than it is 101% from 100%.

The half life of ^{45}Ca is 165 days and of ^{32}P is 14.3 days and both emit β^- particles. These particles only travel short distances through bone and soft tissues and any concentration of radioactivity could only be registered by a sensing device within a 1 cm. range. Isotopes emitting γ (gamma) rays, which are not significantly stopped by soft tissue, can be detected at a greater distance and are much more useful in man and living animals. For γ radiation, the sensing device can be applied to the body surface or even some distance away, i.e. a non-invasive measuring technique can be used.

The first isotope to be used for bone in this manner was not of calcium but an element with similar chemical behaviour, strontium. The isotope used was ^{85}Sr, which has a half life of 65 days, giving rise to a very unstable γ emitter. The emission was detected by scanning with a rectilinear scanner or scintigraphy using a gamma camera. The technique proved useful in detecting metastatic skeletal tumour deposits not visible radiologically. It was therefore used for patients with caisson disease of bone (Cox and Walder, 1976). Positive scans were found in fourteen out of seventeen juxta articular lesions and none out of three shaft lesions. One femoral head and four humeral head had positive scans, using the opposite limb as a control, in the presence of a normal radiograph.

The resolution of scans with ^{85}Sr is restricted by its high γ energy and the small dose which can be safely used (because of the long half-life and high energy). Cameron (1969) found that other nontraumatic osteonecrosis of the femoral head also had increased uptake of ^{85}Sr.

Because of the limitations of ^{85}Sr, a large number of other isotopes have been investigated. Calcium -47 (^{47}Ca) is an emitter with a suitable half-life (4 - 7 days) but very high energy emissions. If produced by meutron irradiation in the pile of enriched ^{46}Ca, rather than by proton irradiation in the cyclotron of enriched ^{48}Ca, the ^{47}Ca tended to be contaminated with ^{45}Ca, which has a half-life of 152 days (Bauer and Wendeberg, 1959). It was used by Danielsson et al. (1963) to study osteoarthritis in hip joints but because of the high energy the results could only be expressed as a ratio of counts over one joint to counts over a normal joint (the report used hip/knee ratios). Accuracy of diagnosis was poor when compared with radiology and subsequent histology. Radiosodium(^{22}Na and ^{24}Na) and radiogallium (^{72}Ga) proved unsuitable and barium - 140 (^{140}Ba) has only been used in one study of rabbits (MacDonald, 1958) when it was found to behave similarly to ^{85}Sr. ^{87m}Sr has a short half-life but slow clearance from the blood, a difficult pair of characteristics for detecting a focal increased uptake. Greater success has come from using radioisotopes that incorporate into forming hydroxyapatite, which has the formula $Ca_{10}(PO_4)_6(OH)_2$ as cations rather than anions. Fluoride ions will exchange with the hydroxyl residues. The radioisotope used, fluorine - 18 (^{18}F) is produced by cyclotron reactor and has a half-life of 1.85 hours and γ emission of a similar energy to ^{85}Sr. It was shown to have increased uptake in a number of non-malignant focal bone diseases, including aseptic necrosis (O'Mara and Baker, 1973). The results of 18F skeletal scans on six

professional divers with radiologically proven osteonecrosis have been presented by Gorten and Cooley (1974). The scans detected most of the areas of osteonecrosis but bilateral lesions caused problems as there was no unaffected contralateral limb to use as a normal control. In these circumstances only the side showing the greater uptake in that area could be interpretated as being normal. ^{18}F is produced by a cyclotron reactor and its short half-life therefore limits its routine use to centres within easy reach of a cyclotron. A major advance was the linking of technetium - ^{99m} (^{99m}Tc) to inorganic phosphates which would absorb on to forming hydroxyapatite. ^{99m}Tc is produced during the decay of molybdenum - 80 (^{80}Mo) which could be made widely available also 99mTc has a half-line of 6.1 hours and an ideal γ energy, low enough to produce good localisation by external counting methods and giving a reduced dose of radiation to the subjects. Because of this it had already been used for scanning of the brain and liver, and therefore was available in many medical physics departments.

The original compounds to which ^{99m}Tc was bound for skeletal imaging were long chain polyphosphates (Subramanian and McAfee, 1971) but complexes using pyrophosphates, diphosphonates, and imidodiphosphates soon followed. These compounds have been used extensively in the last few years and as well as the list of benign conditions given in 1973 by O'Mara and Baker, increased uptake of bone-seeking radionuclides has now been reported in stress fractures before radiography shows the completed fracture (Marty et al., 1976), Legg-Perthes' disease (Danigelis, 1976) and myositis ossificans (Suzuki et al., 1974).

It has been possible to show in artificially produced lesions of osteonecrosis in rabbits, where the timing of the bone injury is

clearly defined by the date of operation, that skeletal scintigraphy using a ^{99m}Tc diphosphonate compound can detect increased localisation of radioactivity three weeks later. At this time, radiological examination was always negative, though in some animals radiological changes were seen many weeks later (Gregg et al., 1977a). Although divers and compressed air workers with radiographic lesions have positive scintigraphs it has yet to be reported in human subjects that radionuclide skeletal imaging will detect a lesion before conventional radiology, i.e. that there is a transition over a period from

a. normal scintigraph plus normal radiograph, to

b. abnormal scintigraph plus normal radiograph, to

c. abnormal scintigraph plus abnormal radiograph.

While the earlier detection of bone necrosis might be reason enough to further investigate skeletal imaging in divers and compressed air workers, there is a further factor in its favour. Conventional radiological examination can only be carried out for survey purposes annually because of the dose of radiation received by the subject. It is difficult to reduce dosage; but with radionuclide imaging agents the dose can be reduced and a similar result still obtained by external counting of the decay for a longer time. Thus, if the usual dose of a 99m Tc labelled diphosphonate agent for clinical use was 15mCi of radioactivity, for survey purposes this might be reduced to 10m Ci. It is awkward to directly compare the radiation received by the same subject with radiography and a radioisotope study because the former is localised whereas the latter disperses throughout the body. However, the dosages calculated by Mr. J. Haggith, physicist to the Northern Region Medical Physics Centre, for an application to allow use of radionuclide skeletal scanning on a survey basis are given for information.

	DOSE (m rads)			
	SKIN	GONADS	SKELETON	WHOLE BODY
a. 10mCi ^{99m}Tc	-	210	450	110
b. X-ray Pelvis LS spine and LS joints coned view	3,500	400	-	-

It might, therefore, be permissible to perform skeletal scintigraphy more often than radiography on a survey basis.

The definition of ^{99m}Tc localisation is such that with present diphosphonate compounds and scintigraphic equipment, the outline of individual bones is usually clearly visible. It might, therefore, be possible to see an area of dead bone as an area with no uptake of radioisotope, even earlier than the repair reaction associated with an increased uptake. Unfortunately, dead bone incorporates substantial amounts of bone-seeking radioisotopes, as shown by Ray et al. (1962) and Stevens (1963). The experimental technique involved surgical removal of one tibia, freezing and thawing alternatively three times to cause death of osteocytes, and then implantation of the dead tibia into the abdominal wall. They found an uptake of ^{45}Ca by the cancellous bone of the epiphyses and metaphyses of the dead tibiae which was 25% of that in the corresponding areas of the living tibia from thirty minutes to 24 hours after administration. Similar values were obtained for the aminoacid proline labelled with carbon - 14 (^{14}C.) This period includes the time interval of 2 to 4 hours after administration of ^{99m}Tc when scintigraphy is usually performed.

The pattern of uptake of diphosphonate skeletal imaging agents (as of other bone seeking radionuclides) is maximal in the axial

skeleton with uptake in the limb long bones being concentrated in the epiphyseal and metaphyseal regions. At present, the diphyseal regions of long bones cannot always be clearly distinguished from the background activity of radioisotope still present in the blood stream and soft tissues. This might make an area of decreased activity in the shaft of a long bone difficult to detect. However, if areas of osteonecrosis when they first occur occupy the large areas of femoral and humeral heads indicated by later histology (McCallum et al., 1966) this large area might show a decreased uptake. This would depend on radioisotope imaging being performed on an asymptomatic worker at a time between osteonecrosis developing and new bone formation of the repair process starting. Extrapolating from the report of Gregg et al. (1977) on artificially induced bone necrosis in rabbits, this time interval only lasts two or three weeks.

Review of the literature shows several claims of detection of areas of decreased activity on ^{99m}Tc skeletal scintigraphy. The first is that of Goergen et al. (1974) and includes patients with metastatic tumour and other with bone infarcts. Riggins et al. (1974) produced avascular necrosis of the femoral head of dogs by severance of the blood supply and found a decreased uptake of ^{18}F. They noted that two of the dogs later developed increased uptake and attributed this to revascularisation. Danigelis (1976) recorded abnormally decreased activity in the proximal femoral apiphysis in Legg-Perthes disease but later found 'adjacent zones of increased uptake'. He quotes Ash et al. (1975) as showing this prior to radiographic changes but their published abstract does not mention this.

All these reports are of femoral head lesions. Shaft lesions

developing following marrow infarctions in sickle cell disease are in some ways similar to shaft lesions of caisson disease of bone. However, a sickle cell crisis is painful and skeletal scintigraphy a few days later gave an abnormal 'cold spot' (Goergen et al., 1974). Lutzker and Alavi (1976) collected twelve patients with sickle cell crisis, and nine showed a focal decreased uptake both on skeletal imaging and on marrow imaging (using ^{99m}Tc labelled sulphur colloid as a marrow imaging agent). Repeating the radioisotope studies two to four weeks later showed increased uptake of radioisotope, but the authors felt that this was at the periphery of the involved area, with an area of normal or decreased uptake centrally. Such marrow infarctions in sickle cell disease later show new bone formation as an irregular surrounding rim (see, for example, Moseley, 1963) giving a radiographic appearance very similar to B2 shaft lesions of caisson disease of bone. Therefore, it may well be technically possible to detect areas of decreased uptake in both early shaft and head lesions of caisson disease of bone. Late ischaemic necrosis of other aetiologies have recently been reported to give negative marrow scintigraphy using ^{99m}Tc Sulphur colloid (Meyer et al., 1977). The reasons why no such reports for dysbaric osteonecrosis exist are probably twofold. First, caisson disease of bone is asymptomatic and deliberately choosing to perform scintigraphy in the 'acute phase' is not possible. Even an annual examination coinciding with the present medical and radiographic examinations would be unlikely to be in the two to three weeks of the man's life when an area of decreased uptake might be detectable. Second, all the reports referred to above use a pinhole collimator or some form of magnification to produce high quality scintigraphs of small areas.

This can be done if the patient points to where his symptoms are, but is not possible for surveys of asymptomatic subjects. Though there is no reported trial of marrow imaging agents in caisson disease of bone these reasons would be just as applicable to these agents as to skeletal imaging agents.

From the research point of view, one group of human subjects meriting skeletal scintigraphy might be those suffering from acute decrompression sickness, the assumption being that bone damage might have been caused during the same decompression. The greater incidence of radiographic definite bone lesions in compressed air workers admitting to attacks of acute decompression sickness has already been mentioned in the discussion on aetiology. This may mean that the same men are susceptible to both problems rather than the two lesions happening at the same time. However, this seems to me to be the group of subjects most likely to show the full potential of skeletal imaging in caisson disease of bone. Of special interest would be men who have undergone few decompressions, either men who leave compressed air work or diving after only a few days because of trouble with acute decompression sickness or visitors to compressed air contracts (e.g. older men in a supervisory or managerial capacity) who develop decompression sickness. In these groups both scintigraphic and radiographic findings could be related to bone damage induced during a short time period and this is potentially useful. The earliest radiographic changes at present known developed three and a half months after first hyperbaric exposure, but it has been pointed out that earlier radiological changes might not be expected as osteo-necrosis occuring after fracture or corticosteroid therapy also takes at least that long before radiographic evidence is seen (Jones, 1974).

SUMMARY

Diagnostic ultrasound may be useful in detecting bubbles and preventing acute forms of decompression sickness, especially during decompression from very deep dives. This may also prevent caisson disease of bone.

If abnormal chemical values are found, even of a substance thought to be a specific indicator of bone damage, the site of this damage cannot be determined. The only technique under evaluation for earlier diagnosis which would indicate the site of bone damage is skeletal scintigraphy. It might be useful to follow up abnormal biochemical values observed after decompression with scintigraphic examination. One group of research workers is already performing scintigraphy about ten to fourteen days after episodes of acute decompression sickness, but results are not yet available, and the coincidence of both lesions (acute decompression sickness and caisson disease of bone) is only an assumption.

SECTION TWO

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EXPERIMENTAL WORK

CHAPTER 4 - INTRODUCTION TO EXPERIMENTAL WORK

Present research work in caisson disease of bone can be divided into three:

1. Measurements and observations of decompression - related lesions in man (i.e. in compressed air workers and divers).
2. The production of lesions by decompression of laboratory animals, with observations and measurements during the experiment.
3. The use of simulated lesions (not decompression-induced) in laboratory animals.

There are disadvantages with all these approaches.

Compressed air workers and divers undergo multiple hyperbaric exposures, and decompressions. The occasion when bone damage occurs is not known therefore any test to detect it would have to be regularly repeated and would have a low yield of positive results even if it worked. Neither compressed air workers or divers are the most readily accessible subjects for repeated research investigations. Compressed air workers tend to be a shifting population moving to another contract many miles away when their contract is completed, or if better pay is offered. Commercial divers at present work in greatest numbers from oil-rigs, and certainly most of the saturation diving is done from these rather inaccessible installations with no medical staff. Divers with experience of saturation diving have a greater prevalence of caisson disease of bone (Thickett and Evans, 1977), but are especially difficult to use for research investigations because after completing their time in saturation they are usually airlifted back

to shore for leave by the next available transport. Experimental saturation dives, such as those performed in Naval shore establishments, provide valuable opportunities for research.

It is unethical to produce bone necrosis in volunteers because the juxta-articular lesions are potentially disabling. Also, volunteering for one dive, or simulated dive, may be permissible but repeated dives for a long period will not be as the subject cannot be expected to lose time from normal work. Measurements during a single dive are useful to see if biochemical or other values thought to change if bone necrosis develops also change with exposure to pressure alone, without subsequent bone necrosis.

Other problems with human subjects are the strict limitation of exposure to ionising radiations, especially in people in the normal reproductive age range, and the limitations of invasive techniques, such as blood sampling, to gain subject co-operation.

Laboratory animals can readily be made to experience acute decompression sickness but it has proved very difficult to produce bone necrosis in the same species even after repeated decompressions. Most laboratory animals are much smaller than human adults and have a faster circulation time. Theoretically, this means the blood passes through the pulmonary capillary bed more often to allow gas exchange, but the relevance of this faster circulation time to the inability to produce bone lesions is not clear (Kindwall, 1962).

Perhaps more important with smaller animals is smaller bone size, for example if a rabbit femoral head is made necrotic by sectioning its blood supply it will revascularise in three months. Adult sheep have a femoral head about one-third the diameter of a human femoral head, but complete capsulotomy plus almost complete subcapital osteo-

otomy of the femoral neck led to no lesions in the femoral heads when they were examined histologically 8 to 16 months later (Coltman and Walder, 1976). Animals may have a better blood flow than man to areas susceptible to bone necrosis and quadrupeds may exert less force on any one limb than an erect-standing biped.

A number of investigators have produced bone necrosis by decompression of laboratory animals. These experiments are summarised in Table 2. Findings of two kinds are reported. The first is aeroembolism of the bone marrow after rapid decompression (Colonna and Jones, 1948 ; Antopol et al., 1964; Shim et al., 1967). The decompression profilesused in such experiments are ones which are often fatal, and produce aeroembolism in many tissues and in the circulation. This change seems distinct from bone or marrow necrosis, though marrow surrounding the gas bubbles may appear compressed.

The second is bone and marrow necrosis. Horváth and Vizkelety (1973) used rabbits and claimed to produce necrotic areas in the articular cartilage, growth plate and metaphysis, which are unusual sites for bone necrosis of any aetiology. However, they also say large necrotic areas were found in the territory of the main nutrient artery (presumably diaphysial lesions). Other workers using rabbits have failed to produce bone necrosis. Genetically obese mice are more susceptible to acute decompression stress than normal mice and fibrosis and fibrochondromatous structures were found in the marrow of animals killed four to twelve months later (Antopol et al., 1964). Over 400 obese mice were used by Chryssanthou (1976) and were exposed to a pressure of 75 p.s.i.g. for 2 to 6 hours and a slow decompression lasting 60 - 260 minutes. With this protocol few mice developed decompression sickness but over one third developed bone necrosis on later histological examination. There was a higher incidence in

TABLE 2 - BONE NECROSIS FOLLOWING DECOMPRESSION OF EXPERIMENTAL ANIMALS

AUTHOR	ANIMAL	EXPOSURE *	LESIONS PRODUCED
COLONNA & JONES (1948)	RABBIT	55 psig x 5.5 hrs. RAPID DC.	CYSTIC AREAS IN MARROW NO BONE CHANGES ONE MONTH LATER
LÜBOW (1951) (QUOTED BY WÜNSCHE AND SCHEELE 1973a)	ALBINO RAT	FAST DC	CAUDAL VERTEBRAE AND LONG BONES SHOWED EXTENSIVE STREAKS OF NECROSIS
ANTOPOL et al. (1964)	OBESE MICE	75 psig x 6 hrs. DC 1 min.	FIBROSIS AND FIBROCHONDROMATOUS STRUCTURES IN THE MARROW 4 - 12 MONTHS LATER. OCCASIONAL CYSTIC AREAS IN THE MARROW
SHIM et al. (1967)	RABBIT	135 fsw x 1.5 hours	NO BONE NECROSIS. ONE ANIMAL SHOWED CYSTIC AREAS IN THE MARROW
WÜNSCHE & SCHEELE (1971)	ALBINO RAT	1.0 or 3.0 kp/cm^2 x 70 - 90 min. MULTIPLE EXP. SLOW DC.	CYSTS NEAR JOINTS 14 MONTHS LATER
REEVES et al. (1972)	MONGREL DOG	av 66 fsw	NO BONE NECROSIS
HORVÁTH & VIZKELETY (1973)	RABBIT	2.5 atm x 6 hrs. or 1.0 atm x 5 hrs. DC 42 mins.	WIDESPREAD RADIOLOGICAL & HISTOPATHOLOGICAL CHANGES 3 - 120 DAYS LATER
WÜNSCHE & SCHEELE (1973a)	MINIPIG (aged 2 - 3 months)	71.1 psig x 2 hrs. MULTIPLE EXP. DC 60 mins.	NO RADIOLOGICAL CHANGES. 2 CASES THICKENING OF BONE BY SCLEROSIS. NO CIRCUMSCRIBED BONE NECROSIS. SIX CYSTS.
WÜNSCHE & SCHEELE (1973b)	ALBINO RAT	12 kp/cm^2 'CRITICAL' DC	CYSTS IN EPIPHYSES OF LONG BONES

TABLE 2 - CONTINUED

AUTHOR	ANIMAL	EXPOSURE *	LESIONS PRODUCED
SMITH & STEGALL (1974)	MINIPIG	60 fsw x 6 hrs UP TO 80 EXP. DC 2 mins.	RADIOLOGICAL CHANGES. BONE NECROSIS IN TWO ANIMALS (1 BIOPSY, 1 AUTOPSY.)
COX (1974)	RAT	75 psig x 2 hrs. MULTIPLE EXP. DC 3.5 mins	NO BONE NECROSIS
EGURO et al. (1974)	GUINEA PIG	135 fsw x max. 1.5 hrs. USN TABLE DC.	NO BONE NECROSIS FIVE MONTHS LATER
KUPPER (1976)	SQUIRREL MONKEY	80 fsw x 3 hrs, THEN 15,000 ft. ALTITUDE x 1 hr.	NO RADIOLOGICAL EVIDENCE OF BONE NECROSIS
CHRYSSANTHOU (1976)	MOUSE	75 psig x 2-6 hrs. DC 60 - 260 mins.	HISTOPATHOLOGY 2 - 17 MONTHS LATER SHOWS BONE AND MARROW NECROSIS
GREGG (1977)	MINIPIG	60 fsw x 6 hrs. DC 56 mins.	ONE CASE MACROSCOPIC RADIOLOGICAL & HISTOPATHOLOGICAL BONE & MARROW NECROSIS
GOLDING (1977) (QUOTED BY GREGG (1977))	GOAT	NO DETAILS	NO BONE NECROSIS

* key : psig = pounds per square inch guage pressure
fsw = pressure in feet of sea water
kp/cm^2 = pressure in kiloponds per square centimetre
atm = pressure in excess atmospheres
DC = decompression.

mice who had had multiple exposures. The earliest time histological changes were found was two months after exposure. This report has not been confirmed by other workers, but I suspect this may be because it is difficult to obtain such obese mice. (The mice used had an average weight of 78 gm.) Three groups of workers have used miniature swine. The first to publish their results were Stegall et al.(1973). They exposed 11 minipigs to a pressure of 60 fsw for 6 hours with fast decompression (2 minutes) and repeated this until the minipigs died of decompression sickness or retired. From six weeks to one year later, the minipigs developed radiographic changes interpreted as dysbaric osteonecrosis. Later reports (Smith and Stegall, 1974; Stegall and Smith, 1976) include histology of a biopsy of one radiographic lesion and of post mortem material from another minipig without any radiographic changes. Histology shows dead bone with empty lacunae, marrow fibrosis, and appositional new bone formation. Surprisingly, there is no report of post mortem material from the pig which had a biopsy taken, surprising because the radiographs suggest that a lesion is present that would have been seen macroscopically. Wünsche and Scheele also reported in 1973. In a series of 37 pigs, compressed for 2 hours at 71 psig and decompressed slowly (60 minutes) they found no histological evidence of bone necrosis or processes of cellular breakdown, and no radiographic abnormalities were seen. They report two areas of bone thickening caused by sclerosis, one in a scapula and the other in a femur, and six bone cysts. Perhaps the most convincing report came from the third group. Gregg (1977) subjected four miniature pigs to multiple exposures to a pressure of 60 fsw for 6 hours with a 56 minute decompression. One developed a lesion that macroscopically looked very like a human shaft lesion, and histologically showed bone and

marrow necrosis and new bone formation. The area also had positive radiographic and scintigraphic appearances.

It is interesting that the only reported attempt to use primates (Kupper, 1976) failed to produce radiographic evidence of decompression induced bone necrosis in squirrel monkeys.

As with human beings, when multiple exposures to pressure are used in animals the occasion when bone damage is induced is not known. As even the miniature swine discussed above are much smaller than man most of the bone lesions might repair completely when present for many months. On the other hand, the lesions probably take two to three months until they show radiographically. For either reason, the animals might be sacrificed at an inappropriate time.

Simulated lesions in animals could be produced by surgical interruption of the arterial supply but for technical reasons this would have to be performed on large extraosseous vessels. If gas bubbles are important aetiologically they probably occlude small vessels within the bone. For intravascular bubbles this has been simulated by intra-arterial injection of inert sterile particulate matter, for extravascular bubbles producing vessel occlusion by a pressure effect the intra medullary pressure has been artificially raised. Both these experimental models have been known for many years to produce bone and marrow necrosis.

The intra-arterial administration of bland emboli to occlude the small vessels was first performed by Kistler (1934) who used carbon particles as a 2% solution in physiologic normal saline to embolise rabbit femora. Bone and marrow necrosis were observed frequently, in Kistler's words:-

'The recent infarcts were dark red and poorly differentiated, while the older ones were demarcated from adjacent vascular marrow

by centers of pale grey tissue of the nature of fat, that had margins of a more fibrous texture.' Histological description of these areas note cortical and marrow necrosis with periosteal and endosteal new bone formation and marrow fibrosis and photomicrographs reproduced in the published article show trabecular new bone formation between necrotic and living marrow.

Elevation of intramedullary pressure by infusion of physiologic normal saline was performed by Larsen (1938). At high pressures the marrow became completely necrotic and massive necrosis of bone occurred. The large columes of solution necessary to maintain the pressure tended to cause periosteal elevation and wound dehiscence with subsequent infection.

The first of these techniques was revived by Cox (1973, 1974) using graded glass microspheres as blood emboli. He used adult New Zealand white rabbits and found histological areas of aseptic necrosis in femora excised three weeks or more after operation. This work was repeated in immature rabbits (age 10 weeks) by Spivey and Park (1973, 1974) and variable ischaemia of the hip and upper femur resulted. The radiographs had changes in appearance thought by Spivey and Park to be similar to those in human children in Perthes' disease. The difference in the anatomical arrangement of blood supply to the epiphysis before closure of the epiphyseal growth cartilage (see, for example, Brookes, 1971) was thus emphasised.

This model system of artificially induced bone necrosis has been used by the Medical Research Council decompression sickness team to investigate methods of early detection of bone necrosis. However, its limitations must be stressed. The most severe limitation is the assumption that intravascular emboli are the cause of bone necrosis in compressed air workers and divers. If this assumption is allowed,

and intravascular gas bubbles are held to be the cause of decompression related bone damage, an obvious difference is that emboli of glass microspheres will remain at the site of their arrest within the arterial network permanently. This will not be true of gas bubbles. It might be invalid to use the model for measuring haematological parameters as glass spheres may have a different effect to gas bubbles on the vascular endothelium. For biochemical measurements the effect of glass microspheres in interfering with the blood supply of adjacent soft tissues must be considered. Also, the amount of the substance measured may alter with exposure to pressure as well as bone damage, so alterations that appear to be a useful diagnostic tool in the rabbit model may not be useful for clinical work. Possible species differences may be important; the overall haematological and biochemical response of a rabbit may be very different to that of a human being. The model system involves an operation so each assay must include a similar operation that will not produce bone damage to ensure that observed measurements are not caused by the trauma of operation, the anaesthetic agent used, or blood loss during the operation. For any measurements using the model system, the comparability of the bone lesions of different postulated aetiologies (gas bubbles or glass microspheres) must be borne in mind. By comparability I include not only the histological appearance but the time sequence of the development of the lesions and of their repair. The possible importance of different animal size in this respect has already been mentioned.

Despite these limitations, this model using artificial emboli has proved useful in trying out new ideas. This is especially so for measurements suspected to change soon after bone necrosis develops, as the time of bone damage is known as it is induced by

the operation. Far fewer measurements, therefore, need to be made. Positive results obtained must be checked in human beings, or, if the technique is in any way invasive, or not without risk, first in animals exposed to a decompression stress.

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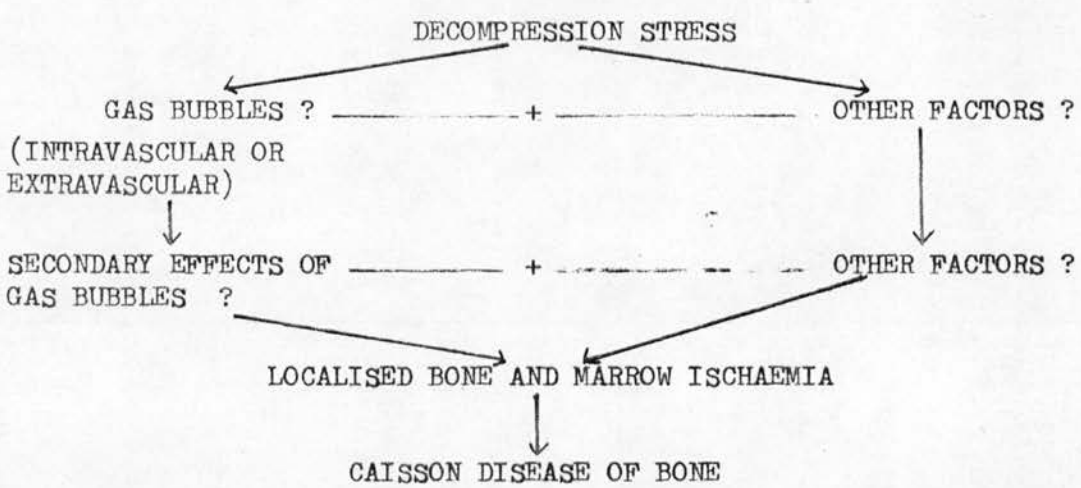
A different division of present research work is into experiments concerned with the aetiology of caisson disease of bone, with its early diagnosis, and with its management. Sometimes an experiment overlaps such groupings.

Experiments on aetiology must somehow break into the proposed sequences outlined at the end of the chapter on aetiology. For the design of experiments these sequences may be simplified to:



I wondered for a long time how to further investigate some of the proposed circulatory changes caused by gas bubbles. For the reasons given in Chapter 2, reports of microscopic findings of fibrin deposition, platelet aggregates, lipid droplets, and erythrocyte accummulations after a rapid decompression have to be interpreted with caution. The only way to study these factors in relation to developing bone necrosis in the living animal seemed to be the use of radioisotope tracers attached to the substances under suspicion.

Unfortunately, even if the radioisotope localised to a particular areas, I could not prove that this was the area where the bubbles were. I then learned of a proposal to administer radioisotope labelled fibrinogen to divers who had suffered from acute decompression sickness to see if it localised in particular bones and might be a possible means of early diagnosis. This assumes fibrin deposition in the circulation in areas of bone and marrow necrosis and it seemed possible that this might be tested using the model system of intra arterial injection of glass microspheres. The extra objections of using this model for measurement of haematological parameters have already been given. Gas bubbles and glass microspheres might not cause similar endothelial damage, and the foreign surface of one might be more or less attractive than the other for the adherence of fibrin, or platelets, or other substances. However, another reason for wanting to perform experiments on animals first is that the substance used for labelling fibrinogen is a radioisotope of iodine and localises in the thyroid gland unless this is therapeutically saturated with non-radioactive iodine beforehand. The half-life of iodine-125, the isotope usually used, is 60 days and the usual clinical dose of 100μCi gives a total body dose of 1.7 x 10-2 rads and a blood dose of 8.2 x 10-2 rads (manufacturer's figures: The Radiochemical Centre, Amersham, England).

I spent a long time investigating changes induced by operative introduction of intravascular glass microspheres in the localisation of ^{125}I - fibrinogen in the hind limbs of rabbits. Unfortunately, any form of intra arterial interference, even an injection of physiologic saline, resulted in a gross elevation of the values recorded and I do not think that it will be possible to reproduce the desired clinical features for investigating fibrin deposition in early bone necrosis in a model system involving an intra-arterial operation.

Another approach to delineate aetiological factors from the subsequent chain of events might be to try and work backwards through the steps of proposed sequences. There are no published reports suggesting that this approach has been investigated before. The last postulated step is that localised areas of ischaemic bone and marrow develop into the typical lesions of caisson disease of bone. The work of Larsen (1938) suggests that marrow ischaemia and necrosis can be induced more easily than cortical necrosis in the shaft of the femur in experimental dogs. This work used infusions of saline to raise the intramedullary pressure. An interesting experiment might be to render ischaemic localised areas of marrow and see if a lesion resembling a shaft lesion of caisson disease of bone developed. This might suggest that the fibrous capsule and new bone formation around shaft lesions was a reaction of the body to the presence of ischaemic or necrotic marrow so that no aetiological factors would need be postulated for this last step in the development of caisson disease of bone. An experimental model for this purpose is described in Experiment I.

Experiments investigating the possible means of early diagnosis form a pattern of trying out ideas in the model system using necrosis artificially induced by intra arterial glass microspheres, then repeating the measurements in animals and man, developing bone necrosis after decompression stresses. The present work includes different stages in the investigation of serum collagenolytic enzymes, 24 hour urinary total hydroxproline excretion, and skeletal scintigraphy, as possible early indicators of caisson disease of bone.

CHAPTER 5 - EXPERIMENTS CONCERNING AETIOLOGY

EXPERIMENT - REIMPLANTATION OF ANOXIC AUTOLOGOUS BONE MARROW INTO RABBIT FEMORA HYPOTHESIS

The idea that bone marrow is potentially osteogenic as well as being haematogenic is well established. If bone marrow is removed from its usual site and implanted into other tissues it survives and a capsule of bone develops around it (Levander, 1940; Lacroix 1951; Danis, 1957). Levander (1940) discussed the earlier literature in detail and found that marrow autografts were uniformly successful in inducing bone whereas marrow homografts never were. He used this observation, together with the evidence from his microscopic examinations of the transplanted marrow, to refute the view that bone formation took place because bone fragments were accidentally transplanted into the subcutaneous layer of 2 year old rabbits. Bone formation was observed in 5 out of 12 animals and Levander writes 'A characteristic feature of the new bone is its peripheral position between the graft and the surrounding connective tissue membrane'. The bone formed was trabecular bone. Lacroix (1951) placed autografts of marrow in young rabbits beneath the renal capsule and observed bone formation in all of 25 experiments.

Pfeiffer (1948), however, transplanted marrow homografts from well inbred mice into the anterior chamber of the eye, and the posterior pole of the testis. Of the grafts subsequently located, 60 or 70 days later, 37 out of 44 had developed bone in relation to the surface of the marrow graft.

More recently rabbits were also used by Nade and Burwell (1977) who found 100% incidence of trabecular bone in marrow

autografts remaining 2-13 weeks after implantation into the rectus abdominis muscle.

Ossification can also be induced in ectopic sites by dead tissues, especially bone decalcified with weak acid (van de Putte and Urist, 1966) and necrotic muscle in the rabbit (Bridges and Pritchard, 1958). Bone formation has even been induced by subcutaneous implantation of a synthetic sponge (Winter, 1970).

Other bone inducing tissues and agents fail to induce bone in the kidney, liver and spleen (in the substance of the organ, not in contact with the capsule) and Chalmers et al. (1975) have suggested that this might be true of bone marrow, at least for the spleen and liver, because marrow is normally present in these sites in foetal life. They point out that it would be a disadvantage if these organs were to therefore ossify.

Bone does not usually form within the marrow space of the medullary cavities of the metaphyses of long bones, unless the space is disrupted by fracture, tumour, or marrow necrosis (as seen in caisson disease of bone and sickle cell disease). The reason for bone formation around areas of marrow necrosis is not known. It might be that necrotic bone marrow is a bone-inducing agent in the same way that necrotic muscle may be. Implanting dead marrow to ectopic sites does not prove this because it is phagocytosed (Danis, 1957). However, re-implantation of marrow autografts into a medullary cavity seems not to have been attempted before.

This would be an interesting experiment in relation to the development of lesions of caisson disease of bone, as it might help to explain how a temporary period of marrow ischaemia might lead to new bone formation and the characteristic radiographic appearance of

a shaft lesion.

MATERIALS AND METHOD

The experiment was performed on adult female New Zealand white rabbits, weighing 3.0 - 5.3 kg. Deep anaesthesia was induced by metered intravenous injection of Pentobarbitone sodium 60mg/ml., ('Sagatal' B. Vet. C., May and Baker Ltd., Dagenham, England). The area over the greater trochanter of the right femur was shaved and the skin prepared with Savlon (1 part : 20 parts methyl alcohol). The rest of the rabbit was covered with sterile drapes to give a sterile field and an aseptic operative technique was employed. The skin was nicked with a pointed blade (Swann Morton No. 11) and a sterile narrow needle and a stilette introduced into the proximal end of the medullary cavity by pushing it through the greater trochanter. This was the part of the operation for which the deep anaesthesia was necessary. The greater trochanter tapers to an acutely-angled ridge in the rabbit but there were no instances of failure to enter the medullary cavity. The stilette was then withdrawn and a glass syringe attached to the marrow needle and marrow withdrawn by sudden suction pressure. 0.5 - 1.5 ml. of marrow were obtained; sometimes, a greater volume than this could have been aspirated but after approximately the first 1.0 ml. the aspirate seemed to be blood with no macroscopic marrow fragments. The aspirate was transferred to a sterile 2 ml. flat bottomed tube, and the stilette was replaced in the marrow needle. The tube containing bone marrow was closed with a cap in which holes had been cut to allow the introduction of a glass pipette and the exit of gas. A sterile pipette was introduced and carbon dioxide gas bubbled through the marrow aspirate for 2 hours

to render it anoxic. The aspirate became less fluid during this period, this could be because of enhanced evaporation due to the throughput of dry gas bubbles, but the volume did not seem to become greatly reduced. The marrow was transferred back to a glass syringe, the stilette again removed from the marrow needle, and the anoxic marrow re-implanted into the medullary cavity of the femur. The stilette was replaced and the needle withdrawn. The rabbit was observed for local bleeding, but no significant blood loss occurred. Until this time the rabbit was kept anaesthetised by small increments of intravenous Pentobarbitone, as required.

Post-operatively, the rabbits showed no ill-effects and used their hind limbs normally, with the exception of one rabbit that developed an unexplained complete paraplegia ten weeks after operation. There was one anaesthetic death and two rabbits subsequently died, one 4 hours after operation and the other three weeks after operation. The rabbits were maintained on BP rabbit diet (BP nutrition (U.K.) Ltd., Stepfield Witham, Essex, England) and water ad libitum until they were killed by intravenous Pentobarbitone overdose. The rabbits were then x-rayed, and the right femora removed, cleaned, and frozen for subsequent sectioning and histological examination.

The bones were deep frozen to allow longitudinal sectioning of the femur without the marrow, which is partly fluid at normal temperature, flowing out of position. This longitudinal sectioning allows much better penetration of fixative into the marrow. The sectioning was performed on a water-cooled rotary diamond saw (Agate and General Stonecutters Ltd., London) and fixation was with 10% formal saline for at least 48 hours. Decalcification was performed with a

mixture of 9 - 10% hydrochloric acid plus ethylene diamine tetracetic acid (EDTA) prepared by the Department of Oral Pathology, The Dental School, University of Newcastle upon Tyne. Decalcification took 24 hours, the bisected bones were then washed in water for 1 - 2 hours, and embedded in paraffin in three parts; head and trochanteric region, shaft, and condyles. 5 - 6 μm longitudinal sections of each part were cut on a Reichert sledge microtome, and stained with Harris's haematoxylin and eosin for microscopic examination.

RESULTS

The procedure was performed on twelve rabbits. In addition, there were four control operations. In the control operations marrow was aspirated in the way already described, but not re-implanted. Instead, normal saline was introduced; the volume used was approximately the volume of marrow aspirated from the same rabbit.

The results of radiographic and histological examination of these animals are summarised in Table 3. Five circumscribed lesions of marrow necrosis with surrounding fibrosis and new bone formation was found.

As early as two weeks after operations, an established lesion, with a well-defined fibrous capsule, was found (Fig. 3). Some areas of this fibrous capsule were differentiating into spicules of bone.

Figure 4 shows a similar lesion lying beneath the cortex in the proximal shaft of the femur of a rabbit killed three weeks after operation. The fibrous capsule appears fairly complete and bone is forming in places.

Figure 5 shows a large area of abnormal marrow, from a rabbit killed six weeks after operation, but the surrounding ring now seems

TABLE 3 - IMPLANTS OF ANOXIC AUTOLOGOUS BONE MARROW

RABBIT	SURVIVAL TIME (WEEKS)	RADIOGRAPHY	HISTOLOGY	
1 : 1	0 (4 hours)	-	Normal. Some large lakes of blood in proximal shaft	NEG.
1 : 2	2	Normal	Extensive lesion base greater trochanter and proximal shaft	POS.
1 : 3	3		Well encapsulated lesion with fibrous/bony capsule	POS.
1 : 4	4	Suspect POS. Sclerotic area mid shaft	Extensive lesion of marrow fibrosis mid shaft	POS.
1 : 5	6	Normal	Ring of fibrous tissue and bone proximal shaft	POS
1 : 6	8	Normal	Normal	NEG.
1 : 7	10		Normal	NEG.
1 : 8	10		Normal	NEG.
1 : 9	13	Normal (poor quality film)	Extensive lesion mid shaft. Surrounding fibrosis and new bone	POS.
1 : 10	13	Suspect POS. Ring (incomplete) of calcification in proximal shaft	Normal	NEG.
1 : 11	17	Normal	Normal	NEG.
1 : 12	17		Normal	NEG.

TABLE 3 - Continued

CONTROL EXPERIMENT : IMPLANT OF SALINE

RABBIT	SURVIVAL TIME (WEEKS)	RADIOGRAPHY	HISTOLOGY	
1 : 13	13	Normal	Normal	NEG.
1 : 14	13	Normal	Normal	NEG.
1 : 15	17	Normal	Normal	NEG.
1 : 16	17	Normal	Normal	NEG.

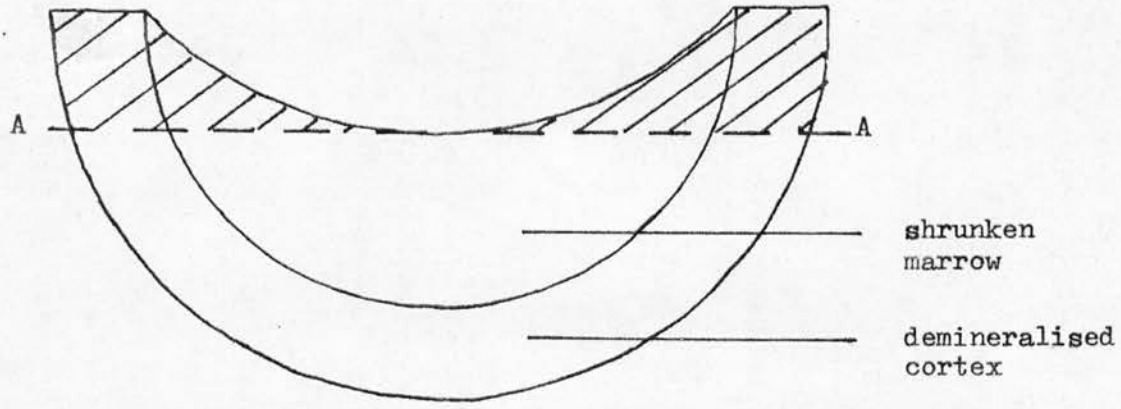
to be predominantly bony (though the bony ring is incomplete) and there is little evidence of fibrous tissue.

Figure 6 is a low power photomicrograph of a large necrotic marrow lesion, in a rabbit killed thirteen weeks after operation. Again there is little evidence of a fibrous ring, but there is a well marked incomplete bony ring. Higher power views of a number of these bony spicules showed occasional resorption lacunae containing multinucleate cells. These had not been noted in the slides from lesions seen at shorter times after operation, even though a careful search was made for them.

Radiographic examination of eight rabbits showed only two suspiciously abnormal films in rabbits killed one month and three months after operation.

In seven histological examinations no lesion or sign of the operation was seen. This may be because marrow shrinkage during processing given embedded half femurs as shown such that sections can only be taken after the line AA, is reached.

CROSS - SECTION of LONGITUDINALLY BISECTED RABBIT FEMUR



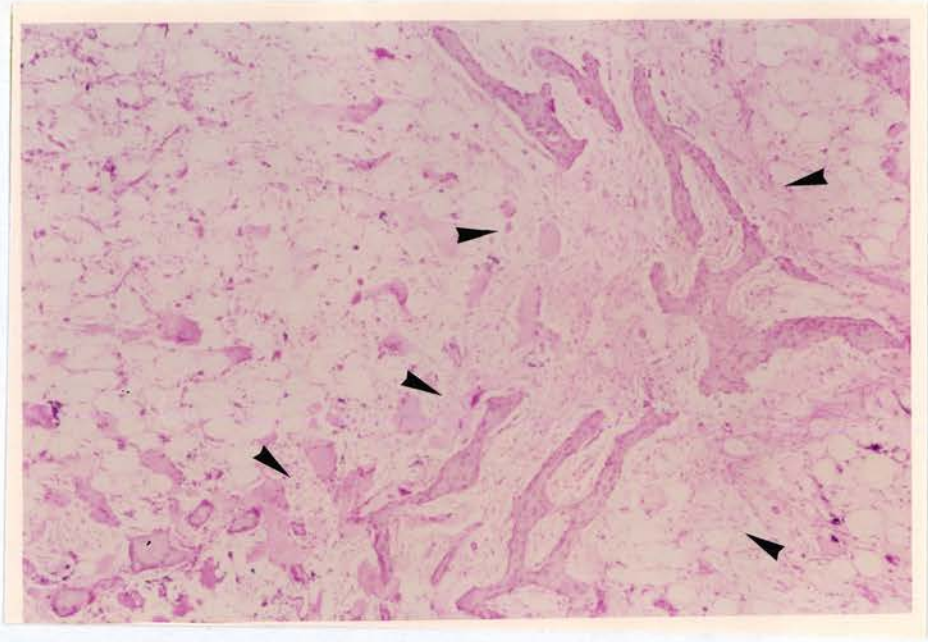


FIG. 3 Lesion 2 weeks after operation. Note the fibrous capsule (between arrows) with enclosed spicules of bone. x 50

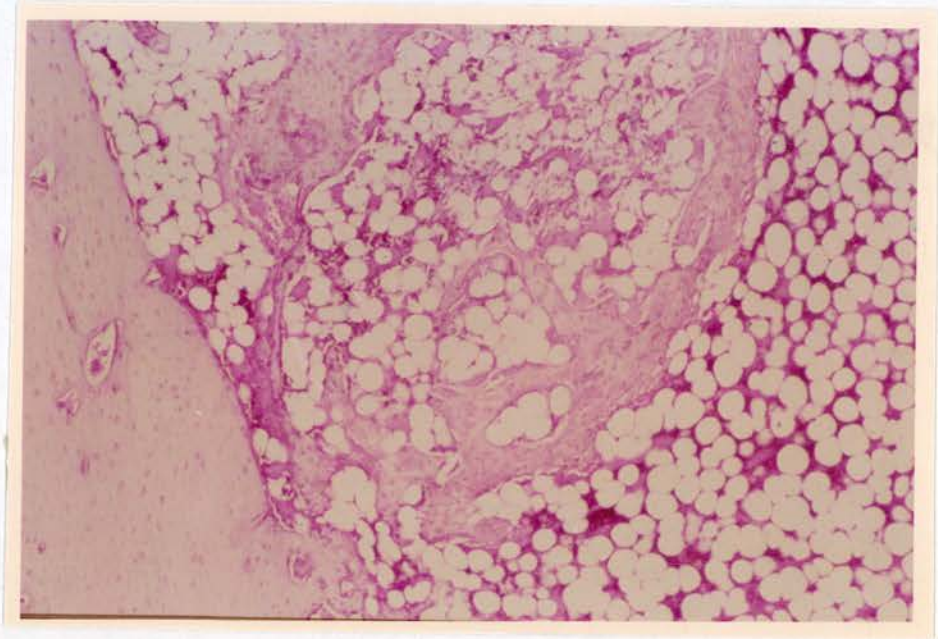


FIG. 4 Lesion 3 weeks after operation. Note the fibrous ring adjacent to the cortex. x 50

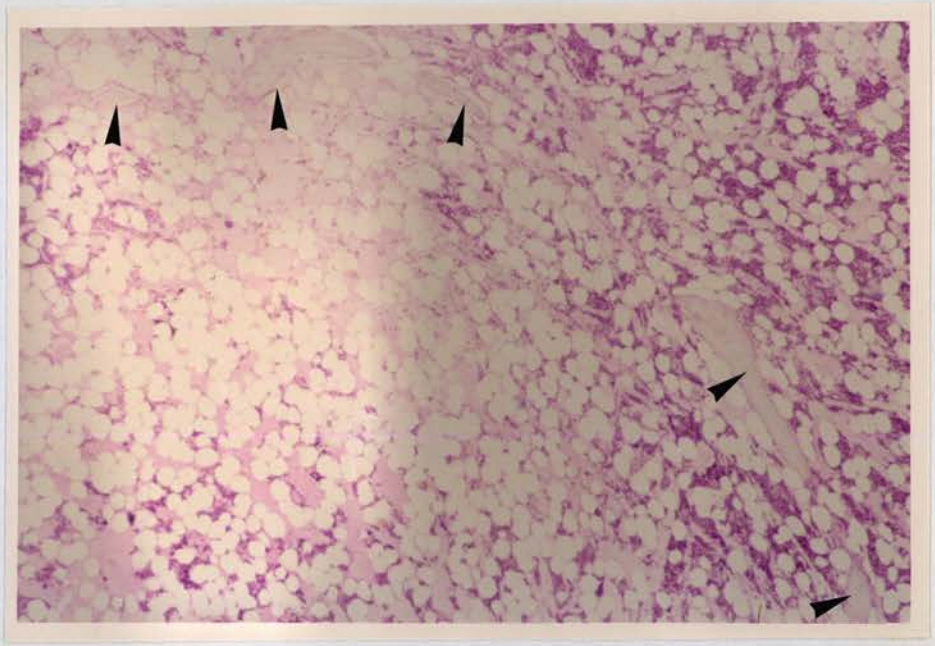


FIGURE 5 Lesion 6 weeks after operation. Necrotic marrow occupies much of the slide, and is surrounded by an incomplete bony shell (arrowed) x 50

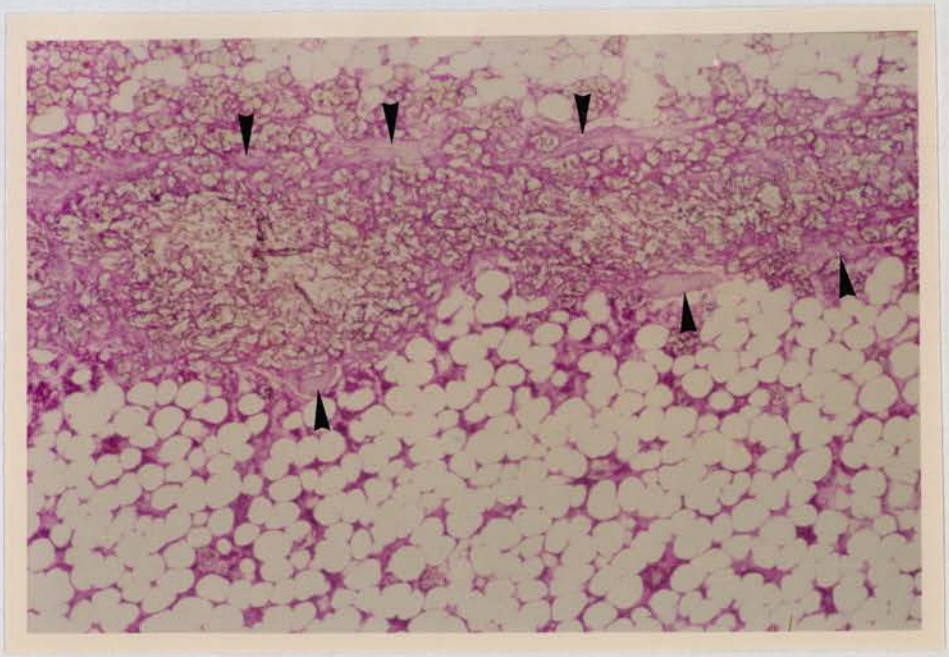


FIGURE 6 Lesion 13 weeks after operation. Note the incomplete bony shell (arrowed) x 50

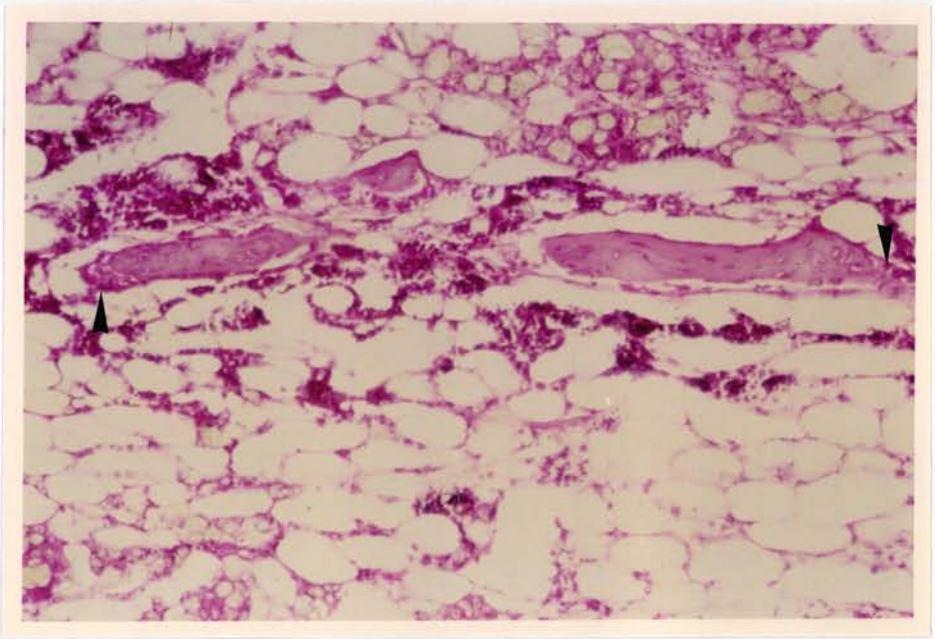


FIGURE 7 Higher power view of the bony shell of lesion 13 weeks after operation shown in FIGURE 6.

Note the multinucleate osteoclasts (arrowed)

x 125

The shaded areas were discarded by the technician because of the central gap in the marrow cavity. However, these rabbits were all eight weeks or longer after operation before death, and any lesions might have already resolved.

DISCUSSION

The results indicate that the re-implantation of marrow rendered anoxic for two hours into the same rabbit femur cause a circumscribed lesion to develop. This has a fibrous capsule with trabecular new bone formation strikingly similar histologically to shaft lesions of caisson disease of bone, and to lesions produced in rabbit femora by arteriolar blockade with glass microspheres. No necrosis of adjacent femoral cortex was seen even when the lesions lay close to the endosteum on the examined histological section. Cortical necrosis was not expected as the operative technique should not have disrupted much of the medullary circulation.

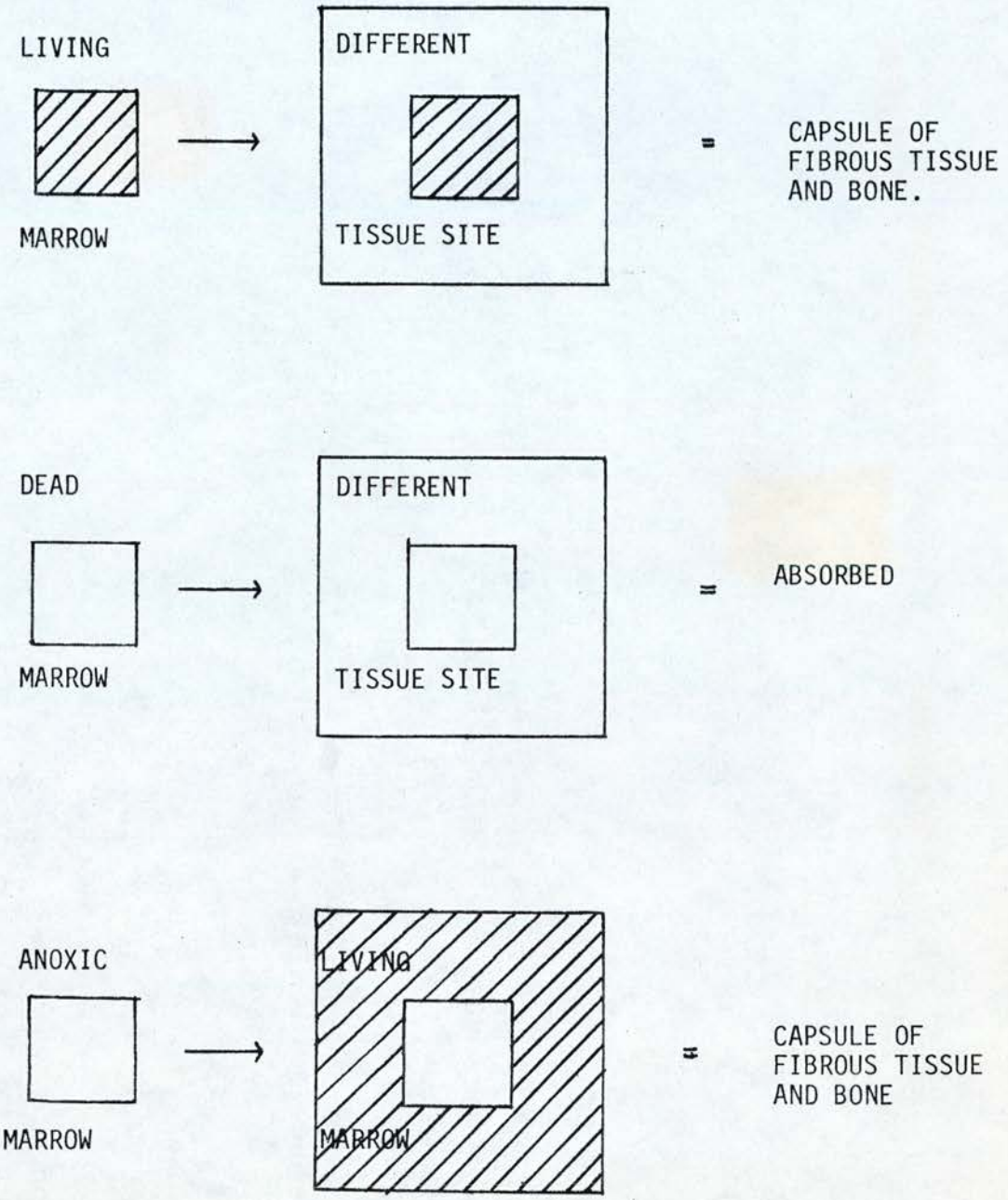
This experiment raises two questions:-

1. Why should the presence of anoxic marrow within the living marrow of the medullary cavity provoke this fibrous tissue and bony reaction?
2. Is it a coincidence that transplants of fresh autologous marrow to ectopic sites cause an apparently similar reaction? Transplants of killed autologous marrow to extopic sites do not form bone (Danis, 1957).

The only common factor, as indicated on Fig. 8, appears to be a surface of living marrow (cross hatched on the figure), if we assume that in the experiment described here, the anoxic marrow remains abnormal so that there is a boundary between it and the living marrow. It certainly appears to remain abnormal histologically. If this is

FIG 8. SUMMARY OF FATE OF MARROW AUTOGRAFTS.

(SEE TEXT).



the correction explanation then the experiment might be repeated with the introduction of an inert foreign material into the medullary cavity to provide a surface when we might expect a fibrous and bony capsule to develop.

As soon as I thought of this experiment, I realised that it was unnecessary. Experimental implantation of a silver tube into a long bone marrow cavity was found to give rise to new bone formation within the tube by four weeks after operation by Ollier (1867). In more recent years Orthopaedic surgeons have implanted many inert materials through and into the medullary cavities of the long bones. These include a multitude of screws, rods, implants as part of joint replacements, and cold curing acrylic cement. Collins (1953) has examined the histological appearance of changes around metal implants and found a thick fibrous wall with thin 'plates' of bone within it. The time course of its development appeared similar to that of fibrous tissue and bone in the described experiment. This reaction is often considered as a reaction to mechanical 'stimulation' but there are certain circumstances where I cannot imagine this would be the case. One such instance would be an intramedullary nail for internal fixation of a fracture of the mid shaft of a femur. The 'tight fit' of the nail preventing angulation is around the fracture and the lower end of the nail lies loose in the medullary cavity. However, the development of angulatory movement at the fracture can be detected radiographically by seeing 'rattling lines' of new bone formation along this loosely fitting portion of the nail.

I feel, therefore, that rather than the anoxic marrow in this experiment promoting the surrounding fibrous tissue and bony reaction, it may be the surrounding living marrow that reacts whenever a new surface is presented to it. Its usual 'surface' is the endosteal

lining of the closed compartment of the medullary cavity, every surface of which is covered by bone. The only time when marrow is found not surrounded by bone is in the foetal liver and spleen. As already suggested by Chalmers et al. (1975) suppressors of osteogenesis must be postulated in the foetal liver and spleen if this hypothesis is made.

Returning to a consideration of the situation in caisson disease of bone, it may be concluded that the last step in the postulated sequence of its development, that from the presence of anoxic marrow to the development of the macroscopic lesion, does not require any external or generalised aetiological factors to be proposed. It may be an inevitable local reaction by the surrounding living tissue. The time interval chosen for marrow anoxia in this experiment was two hours, but it is accepted that the re-implanted marrow would then only obtain oxygen by diffusion, as it would have lost its blood supply and it is not valid to say that occlusion of the microcirculation for two hours (e.g. by gas bubbles) could produce such a lesion. In these circumstances, the circulation is not disrupted by operation and might be expected to return to the anoxic area much more quickly. It may be disrupted by other means, but consideration of this will be deferred until the general discussion.

CONCLUSIONS

Re-implantation of autologous marrow rendered anoxic for two hours into rabbit femora produced a lesion of abnormal marrow with a surrounding ring of fibrous tissue and bone in five out of twelve experiments.

CHAPTER 6 - EXPERIMENTS CONCERNING EARLY DIAGNOSIS

EXPERIMENT 2 - ESTIMATION OF SERUM PROLINE IMINO-PEPTIDASE ACTIVITY FOLLOWING ARTIFICIALLY INDUCED OSTEONECROSIS

HYPOTHESIS

The experimental model of Cox (1973, 1974) used arteriolar blockade to produce bone and marrow necrosis in rabbit femora. Weatherley et al. (1977a) was able to show that such necrosis was accompanied by a significant increase in the 24 hour total urinary hydroxyproline excretion. This was first evident 5 days after operation.

Because of the theoretical advantages of measuring serum collagenolytic enzymes as an indicator of an increased rate of collagen breakdown, especially in man in whom the urinary hydroxyproline excretion varies with diet (as discussed in Chapter 3), it seemed important to repeat the experiment described by Weatherley et al. (1977a) measuring the serum proline imino-peptidase levels pre-operatively and 10 days post operatively.

Simultaneously, measurements were also made in animals undergoing hyperbaric exposures and in men undergoing hyperbaric exposures. The human exposures were part of a series of experimental dives at the Admiralty Marine Technology Establishment. Early results showed no significant changes in four divers undergoing a 300m saturation dive. (Admiralty Marine Technology Establishment, 1978).

MATERIALS AND METHODS

The experiment was performed on adult female New Zealand white rabbits weighing 3.5 to 5.0 kg. The rabbits were anaesthetised with

veterinary pentobarbitone sodium ('Sagatal'; May and Baker) administered intravenously into the marginal vein of one ear. The marginal vein of the opposite ear was then used for withdrawing the pre-operative blood sample. The dose of anaesthetic used was approximately 20 - 35 mg/kg. according to the individual response of the animal. Spontaneous respiration was allowed throughout the operative procedure.

Each rabbit was then placed supine on the operating table with its hind limbs secured by tapes. The abdomen was shaved and cleaned with Savlon (1 part : 20 parts methyl alcohol). The rest of the animal was covered with sterile drapes and an aseptic operating technique was used.

An oblique skin incision was made overlying the right iliac artery and continued in the same line through all layers of the abdominal wall. Loops of small intestine, and the bladder when necessary, were retracted behind gauze swabs by two Langhenbeck retractors, at right angles (one retracting cephalad, the other to the left side). The iliac vessels were dissected free of fat, and a sterile injection of 0.08 - 0.10 ml. glass microspheres 50 - 70 μm diameter (sieved from assorted spheres supplied by Jencons Scientific) in 0.9 ml. normal saline injected into the right iliac artery from a 1 ml. tuberculin syringe with a tightly fitting 23 gauge needle (Sabre; Gillette). The needle was curved before introduction into the artery so that the injection could be directed down the artery in the direction of blood flow. The microspheres could usually be seen passing down the artery in the flowing blood as no attempt was made to occlude the blood flow. Weatherley et al. (1977a) had occluded the superficial femoral artery to try and direct the microspheres into the deep vessels supplying the femur but this had been found by Gregg (1977) to be unnecessary

for the successful production of bone and marrow necrosis and was therefore omitted. The needle was then withdrawn and haemostasis secured by gentle direct pressure with a gauze pledget held in artery forceps. The abdominal wall was closed with black silk in two layers, one to the muscles and the other to the skin, and the animals allowed to recover. No antibiotic cover was used as infection had not been a problem for previous workers. There were no cases of wound infection. Control animals had a similar operative procedure, except that the intra-arterial injection was of sterile normal saline only, the glass microspheres being omitted.

The animals were returned to their cages and maintained on B.P. rabbit diet (B.P. nutrition (U.K.)) and water ad libitum. Shortly after operation successful intra-arterial injection of microspheres was checked radiographically. A typical radiograph showing the distribution of glass microspheres is reproduced in Figure 9.

Blood samples were withdrawn from the marginal ear veins, on post-operative days 1 - 5, 7, 10 and 14. The samples were centrifuged as soon as possible after withdrawal, the serum collected by pipetting and the samples stored at - 20°C. until assayed.

The assay for serum proline imino-peptidase (PIP) acticity was performed using the technique described by Whiteley et al. (1976) and substrate and hydrolytic product supplied by the Fluka Chemical Company (importer: Fluorochem Ltd., Glossop, Derbyshire) by the hormone assay laboratory of Clin Path Services Ltd. (High Wycombe, Bucks, England).

RESULTS

Sixteen rabbits underwent operation. Nine had an intra-arterial injection of glass microspheres and all were found to have large areas

FIGURE 9 - Typical radiograph showing the distribution of glass microspheres after injection into the rabbits right iliac artery.

of bone and marrow necrosis when sacrificed 6 weeks later. Six rabbits served as controls and had an intra-arterial injection of normal saline only. None of these control rabbits developed bone and marrow necrosis, the rabbits again being sacrificed 6 weeks later after operation for histological examination (Table 4). One rabbit died during operation.

The results of serum PIP measurements are given in Table 5 and summarised in Figure 10. Elevation of serum PIP levels did not occur in the rabbits developing bone and marrow necrosis, either individually or as a group. If anything, the rabbits developing osteonecrosis tended to have lower serum PIP levels in the third to seventh days after operation, though these variations have no statistical significance.

DISCUSSION

The rabbit model system used for this experiment showed a significant increase in the 24 hour total urinary hydroxyproline excretion (Weatherley et al., 1977a)and in serum ferritin levels (Gregg et al., 1977b). The serum samples collected from the rabbits used in this experiment are also being assayed for serum ferritin but the results are not yet available. The idea was to try and determine which assay was worthy of further study. Because of the results obtained, it would appear that the serum PIP is not a sensitive enough assay to detect collagen breakdown caused by bone marrow necrosis in one femur (in rabbits). This would accord with the findings of Whiteley et al. (1976) in human subjects with Pagets' disease. They found that there remained an overlap with the normal range of serum PIP levels and that elevated values correlated with elevated plasma alkaline phosphate levels. No change in plasma alkaline phosphatase activity was detected following the operation described in this experiment (Gregg and Walder, 1977).

TABLE 4 - HISTOLOGICAL FINDINGS IN RABBITS FOLLOWING INTRA-ARTERIAL INJECTION OF GLASS MICROSPHERES

R.	HISTOLOGY (Positive findings only)
1.	Patchy osteocyte loss throughout femoral head. Dead trabeculae with appositional new bone formation in proximal femur. One area of marrow fibrosis in the proximal femur and another in the distal shaft.
2.	Femoral head necrotic. Large bone and marrow infarct at base of greater trochanter with signs of repair and new bone formation including a marked periosteal reaction on the upper shaft. Remnants of growth plates still present.
3.	Small area of marrow fibrosis at base of femoral neck. Marrow infarct in distal shaft with patchy cortical necrosis and a marked periosteal reaction.
4.	Marrow infarct containing several necrotic trabeculae at base of femoral neck. Considerable osteocyte loss in one cortex of distal shaft with adjacent marrow fibrosis but no new bone formation.
5.	Femoral head necrotic. Patchy osteocyte loss in one cortex of distal femoral shaft.
6.	Femoral head necrotic with appositional new bone formation. Massive marrow infarct in the proximal femur with much endosteal and boundary zone new bone formation surrounding it.
13.	Died during operation. No histological examination.
14.	An area of periosteal and endosteal reaction with new bone formation in one cortex (showing some osteocyte loss) about mid shaft.
15.	One third of femoral head necrotic. Virtually complete osteocyte loss in proximal two-thirds shaft cortex.
16.	Capital epiphysis completely necrotic. Epiphyses not fused.

CONTROL RABBITS

R.	HISTOLOGY (Positive findings only)
7.	Normal
8.	Normal
9.	Normal
10.	Normal
11.	Normal
12.	Normal Epiphyses not completely fused.

TABLE 5 - RABBIT SERUM PROLINE IMINO PEPTIDASE LEVELS

All values in mU/ml/min

OPERATED GROUP

RABBIT NO.	DAY 0 (BEFORE OPERATION)	DAY 1	DAY 2
1.	4.57	5.15	4.86
2.	4.02	5.58	4.72
3.	2.37	2.15	2.56
4.	3.61	5.31	4.38
5.	5.27	5.35	4.08
6.	2.43	1.89	2.24
14.	1.65	1.79	1.65
15.	0.75	1.45	1.41
16.	3.46	3.64	3.18
Mean $\pm$ S.D	3.13 $\pm$ 2.09	3.59 $\pm$ 3.15	3.23 $\pm$ 1.76

CONTROL GROUP

RABBIT NO.	DAY 0	DAY 1	DAY 2
7.	3.86	3.68	3.16
8.	5.24	3.34	3.53
9.	3.78	3.04	3.12
10.	3.19	2.65	3.48
11.	4.00	3.66	3.17
12.	4.08	4.24	5.42
Mean $\pm$ S.D	4.02 $\pm$ 0.45	3.43 $\pm$ 0.31	3.65 $\pm$ 0.79

OPERATED GROUP

RABBIT NO.	DAY 3	DAY 4	DAY 5
1.	3.55	2.73	2.62
2.	4.15	3.67	3.64
3.	1.13	1.57	1.24
4.	3.22	3.36	2.70
5.	3.25	3.37	3.31
6.	1.63	1.66	1.06
14.	0.91	0.81	0.75
15.	0.55	0.56	0.53
16.	2.54	2.70	2.50
Mean $\pm$ S.D	2.33 $\pm$ 1.70	2.27 $\pm$ 1.33	2.04 $\pm$ 1.34

TABLE 5 - CONTINUED - RABBIT SERUM PROLINE IMINO PEPTIDASE LEVELS

CONTROL GROUP

RABBIT NO.	DAY 3	DAY 4	DAY 5
7.	3.11	-	4.10
8.	3.66	3.90	3.92
9.	3.42	3.20	2.77
10.	3.03	2.31	2.16
11.	3.25	3.15	3.12
12.	4.89	3.45	3.05
Mean $\pm$ S.D	3.56 $\pm$ 0.48	3.20 $\pm$ 0.34	3.19 $\pm$ 0.52

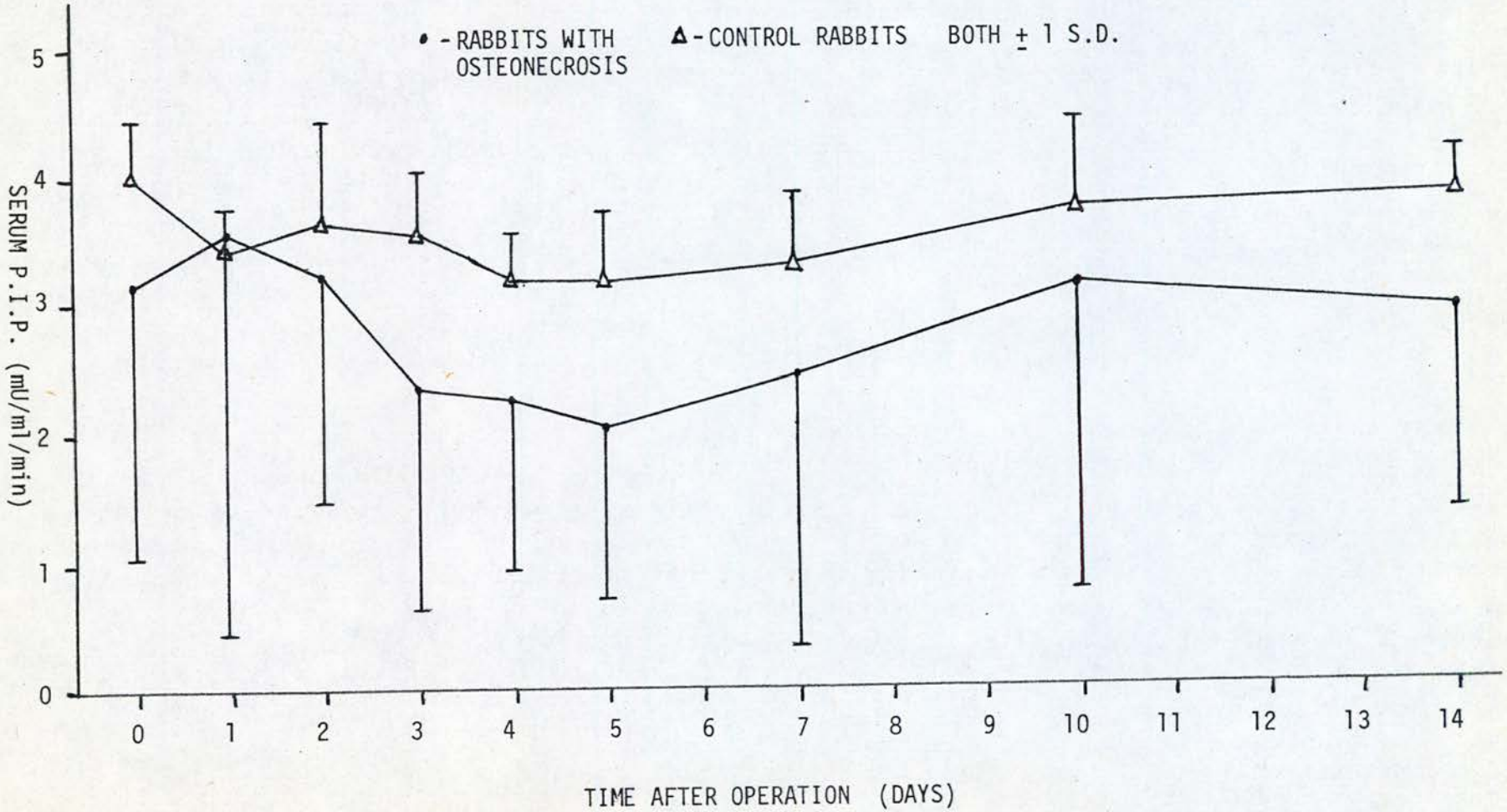
OPERATED GROUP

RABBIT NO.	DAY 7	DAY 10	DAY 14
1.	2.35	3.32	3.91
2.	4.45	5.39	-
3.	1.55	1.92	2.11
4.	3.18	4.77	3.15
5.	4.53	4.72	4.25
6.	1.54	1.94	1.81
14.	0.98	1.12	1.09
15.	0.44	0.99	-
16.	2.99	3.88	4.02
Mean $\pm$ S.D	2.45 $\pm$ 2.13	3.12 $\pm$ 2.33	2.91 $\pm$ 1.54

CONTROL GROUP

RABBIT NO.	DAY 7	DAY 10	DAY 14
7.	3.49	3.58	3.55
8.	4.55	5.24	4.97
9.	2.94	3.32	3.33
10.	2.26	2.79	3.62
11.	3.25	3.57	3.67
12.	3.29	3.98	3.88
Mean $\pm$ S.D	3.30 $\pm$ 0.56	3.75 $\pm$ 0.69	3.84 $\pm$ 0.34

FIG. 10 SERUM PROLINE IMINO PEPTIDASE LEVELS IN RABBITS FOLLOWING INTRA-ARTERIAL INJECTION OF GLASS MICROSPHERES



Because of technical difficulties in obtaining repeated daily blood samples from rabbit marginal ear veins, some of the samples were slightly haemolysed. Any haemolysis was recorded alongside the result, but visual inspection did not show any difference from samples without haemolysis.

CONCLUSION

There was no significant change in the serum proline imino peptidase levels in nine rabbits in the fourteen days following an intra-arterial injection of microspheres, even though bone and marrow necrosis developed in all nine animals.

EXPERIMENT 3 THE EFFECT OF REPEATED EXPOSURE TO EXCESS PRESSURE ON THE GÖTTINGEN MINIATURE PIG

HYPOTHESIS

The effect of exposure to excess pressure and of decompression has been studied in many species and while acute forms of decompression sickness can usually be produced (especially by rapid decompression) there has been a singular lack of success in producing lesions in the long bones resembling those seen in caisson disease of bone in humans. The reports are summarised in the introduction to the experimental work (Table 2). Three separate groups of workers have reported the use ofminiature swine for such studies. Göttingen miniature pigs were chosen for the present study, the aim of which was to attempt to produce osteonecrosis by repeated hyperbaric exposure and to monitor all parameters which had already been suggested as potentially useful indicators of caisson disease of bone. The results of these measurements are reported in experiments 4, 5 and 6.

In this experiment I wish to outline the exposure and decompression profile used and record its observed effects.

MATERIALS AND METHODS

The experiment used 6 Göttingen miniature pigs. On arrival these were castrated males 4 to 8 months old and weighing 12.5 to 20kg. Castration had been performed shortly after birth. The animals were purchased from the Royal Veterinary College which maintains a breeding colony.

As Wünsche and Scheele (1973a) had stated that the marrow of the femur of these minipigs becomes fatty around 12 months of age none of

the animals were exposed to pressure before this age had been achieved. They were maintained on Breeding Sow Cobs (Farmway feeds, Morpeth, Northumberland) and water ad libitum before, during, and after the experimental work. The animal house temperature was set at 21°C., thermostatically controlled.

Starting at 12 months of age, each minipig was given repeated exposures to a hyperbaric environment. The aim of the study was to use an exposure and decompression profile known to give rise to subsequent caisson disease of bone in humans. The one selected was to use the mean working pressure and decompression table used during the construction of the Clyde tunnel in 1958 - 1963. This large tunnelling contract producing an almost 20% incidence of caisson disease of bone in the compressed air workers (McCallum et al., 1966). The pressure selected for the minipig experiment was 27 pounds per square inch guage (27 p.s.i.g.) equivalent to 60 feet of sea water, 1.83 bar, or 1.81 atmospheres. The decompression table used was that of the Work in Compressed Air (Special Regulations) 1958. The compression chamber had internal dimensions 2.59m length x 2.14m diameter main chamber and 1.83m length x 2.14m diameter entry and exit lock. The volume of the main chamber was $9.35m^3$ and that of the entry and exit lock $6.5m^3$. The shift length during construction of the Clyde tunnel was 8 hours, but when this was attempted with two minipigs one collapsed only 12 minutes after reaching the surface after his second full shift and required emergency therapeutic recompression. An exposure time at 27 p.s.i.g. of 6 hours was therefore used, which was the same as the maximum shift length used by Stegall and Smith (1974), and Gregg (1977).

The first exposure in compressed air was for a half-shift only as this is done on compressed air contracts to aid'acclimatisation', that is, to lower the incidence of acute decompression sickness in men

starting work in compressed air. After this the pigs underwent a 6 hour shift each day the chamber was available Monday to Thursday and sometimes a half shift on Friday also. The pattern of exposure for each of the six pigs is shown in Figures 11 and 12.

All of the six minipigs developed symptoms and signs of acute decompression sickness. Before any physical signs developed the affected animal usually refused food and often seemed lethargic. The commonest form of decompression sickness was skin mottling due to vascular stasis. This was especially common in minipig Ollie (Fig 14). Respiratory problems were the next most frequent sign, starting with an increasing respiratory rate (up to 120 respirations/minute) and progressing to a dusky cyanosis and frothing at the mouth. Vomiting sometimes occurred in addition. Untreated episodes could rapidly progress to collapse and convulsions. On only one occassion was limb pain such that the minipig squealed and lifted a leg from the ground, but on several occassions minipigs who were obviously unwell after decompression had tenderness on palpation over a single limb but not the other three. The general impression was that musculo-skeletal forms of acute decompression sickness were much less common in the minipig than respiratory forms, which is the reverse of the findings in humans. Only one minipig, Jerry, developed vertigo. This was after the first pressure decrease following a therapeutic recompression (for acute decompression sickness in a difficult minipig) when Jerry kept shaking his ears and head and falling to his right side when trying to stand. Recompression to the previous pressure was successful in relieving all observable abnormalities and the subsequent decompression was uneventful.

Because it was proposed that bubbles were required for bone

FIG. 11 HYPERBARIC EXPOSURES OF THE SIX MINIATURE SWINE STUDIED IN EXPERIMENTS 3 - 6

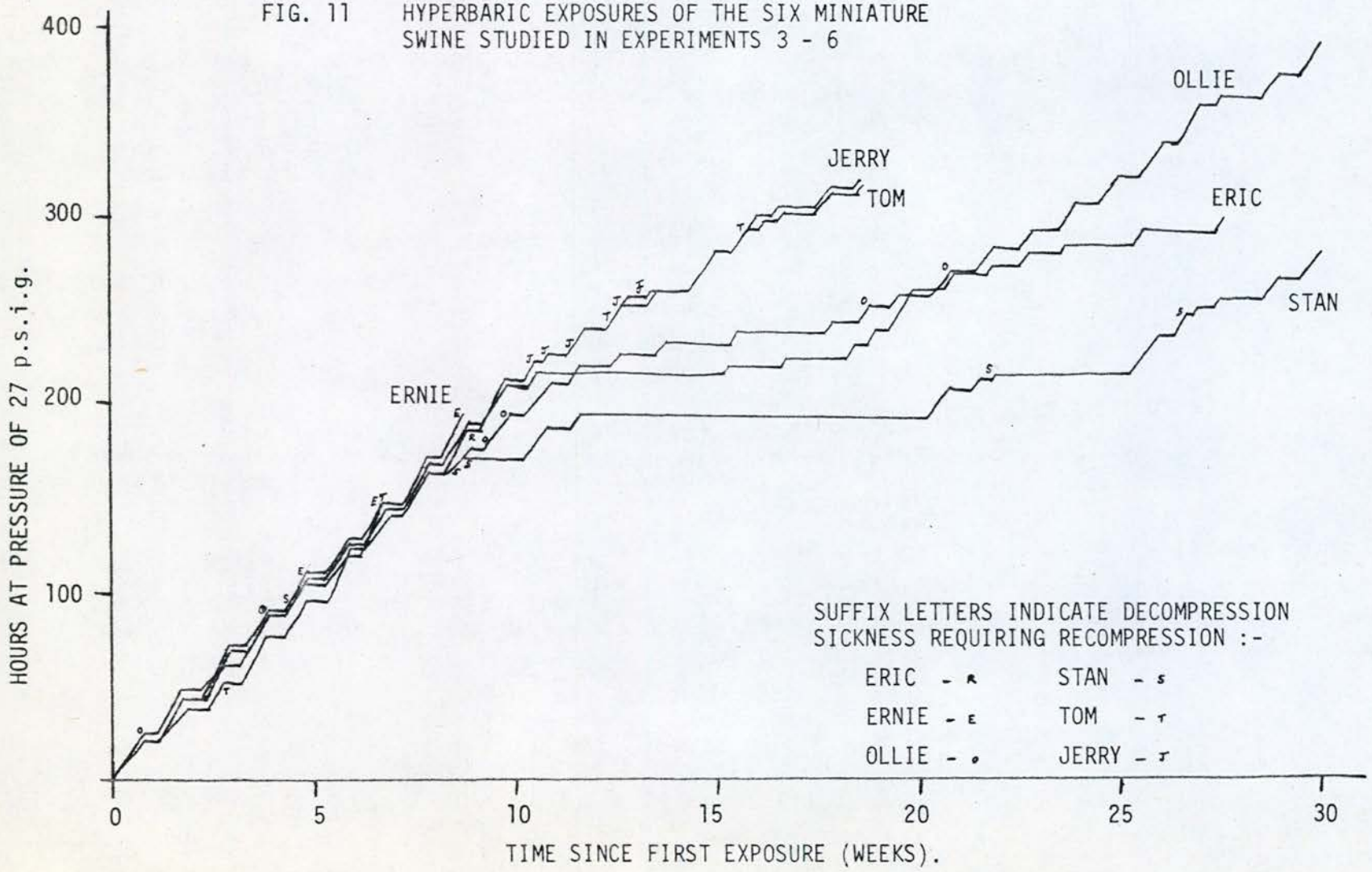
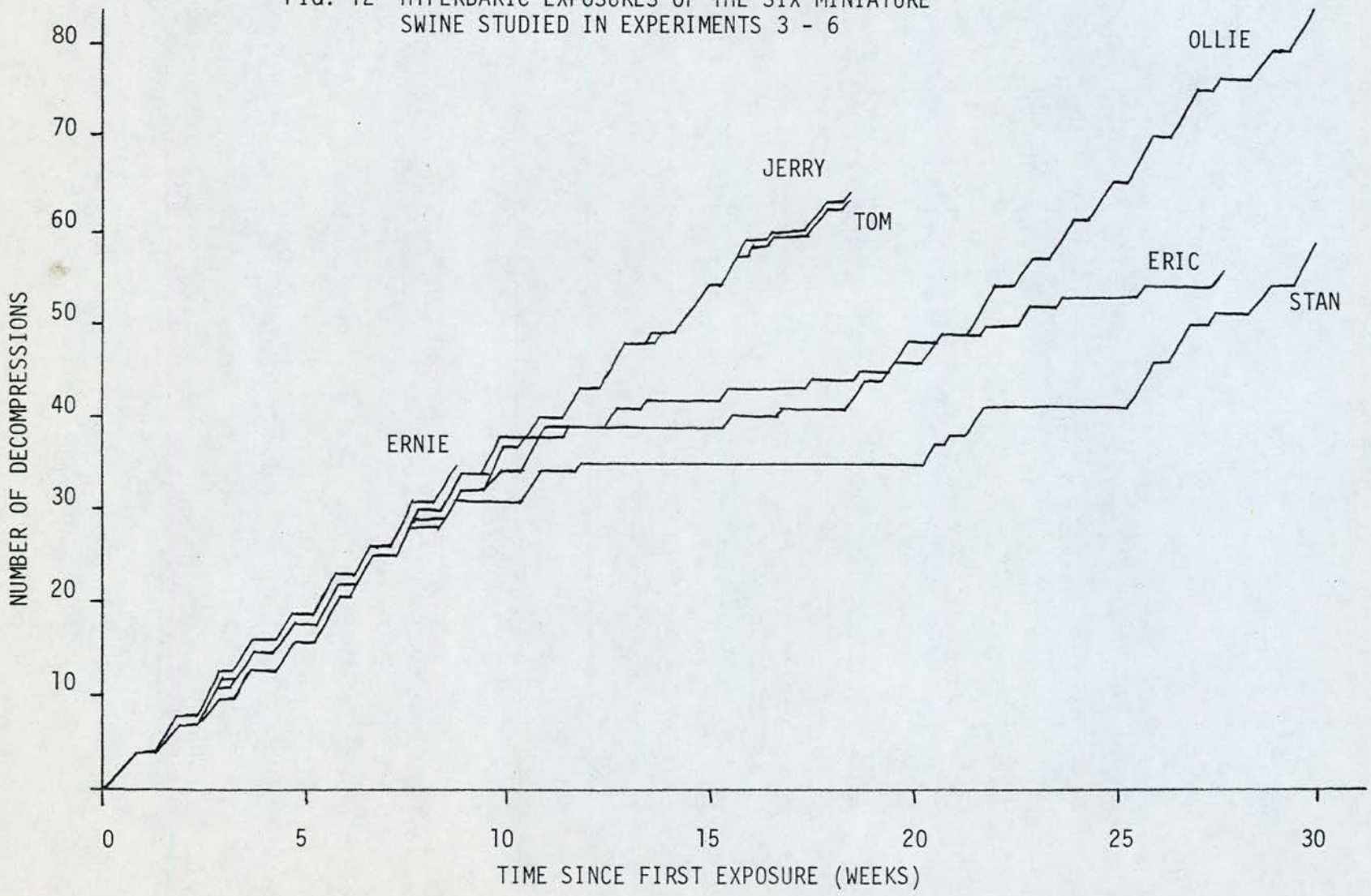


FIG. 12 HYPERBARIC EXPOSURES OF THE SIX MINIATURE SWINE STUDIED IN EXPERIMENTS 3 - 6



necrosis to develop, acute decompression sickness was not treated unless it appeared life-threatening or the animal was obviously in pain. As minipigs with respiratory symptoms and signs could collapse quite rapidly these episodes were often treated by emergency therapeutic recompression, especially if signs developed shortly after the completion of decompression or if signs seemed to be progressing. All minipigs were observed for signs of decompression sickness for at least one hour after the completion of decompression and animals which seemed at especially susceptible periods were often observed much longer before being returned from the chamber to their pens.

If, after a particular exposure to pressure, one of a group of minipigs developed decompression sickness requiring recompression, all the minipigs decompressed at the same time were recompressed if the time was within one hour of the end of decompression, even if the others showed no signs of decompression sickness. The therapeutic recompression was as follows:-

Rapid return to previous maximum pressure (60 f.s.w.)

60 f.s.w.	60 min. "soak"	Each 10 f.s.w. pressure drop lasting 3 min.
50 f.s.w.	30 min.	
40 f.s.w.	30 min.	
30 f.s.w.	60 min.	
20 f.s.w.	60 min.	
10 f.s.w.	120 min.	
Surface		

No recurrence of decompression sickness was observed after this therapeutic recompression.

TABLE 6. THERAPEUTIC RECOMPRESSIONS OF MINIATURE SWINE.

MINIPIG	SHIFT 1 *	SHIFT 2	SHIFT 3	SHIFT 4
ERIC	-	-	-	-
ERNIE	-	-	2	1
STAN	3	-	1	1
OLLIE	3	-	2	1
TOM	1	-	1	2
JERRY	3	1	-	1

* If a minipig had a day off after a recompression and developed acute decompression sickness requiring recompression on the next shift this is counted as Shift 1 because of the day without hyperbaric exposure.

RESULTS. 6 minipigs were studied.

Several were noticed to develop a skin bend after their second or third shift of the week and proceed after the next day's shift to a respiratory bend requiring emergency recompression. The number of therapeutic recompressions required for each minipig for each shift of the week (not every week's shift started on a Monday because the compression chamber was subject to other commitments) is shown in Table 6. Minipigs were excluded from the shift the day after acute decompression sickness requiring recompression.

The progress of individual minipigs will now be considered in more detail.

Minipig Ernie

Ernie commenced hyperbaric exposure at twelve months of age. He required recompression for respiratory distress after 19 decompressions (106 hours at 27 p.s.i.g.), 25 decompressions (140 hours at 27 p.s.i.g.) and 35 decompressions (194 hours at 27 p.s.i.g.). The morning after this last episode Ernie remained unwell with shallow rapid respirations 80/min. and a dusky cyanosis on minimal exertion. Food was refused. This continued and was not affected by a further recompression. The minipig was found dead in his pen 27 days after this last decompression. For the preceding 20 days he had on occasions been seen to be carrying his right foreleg off the ground. Post-mortem showed greatly congested lungs with diffuse bronchopneumonia. The florid changes appeared macroscopically almost to have progressed to something akin to caseation in many places.

Minipig Eric

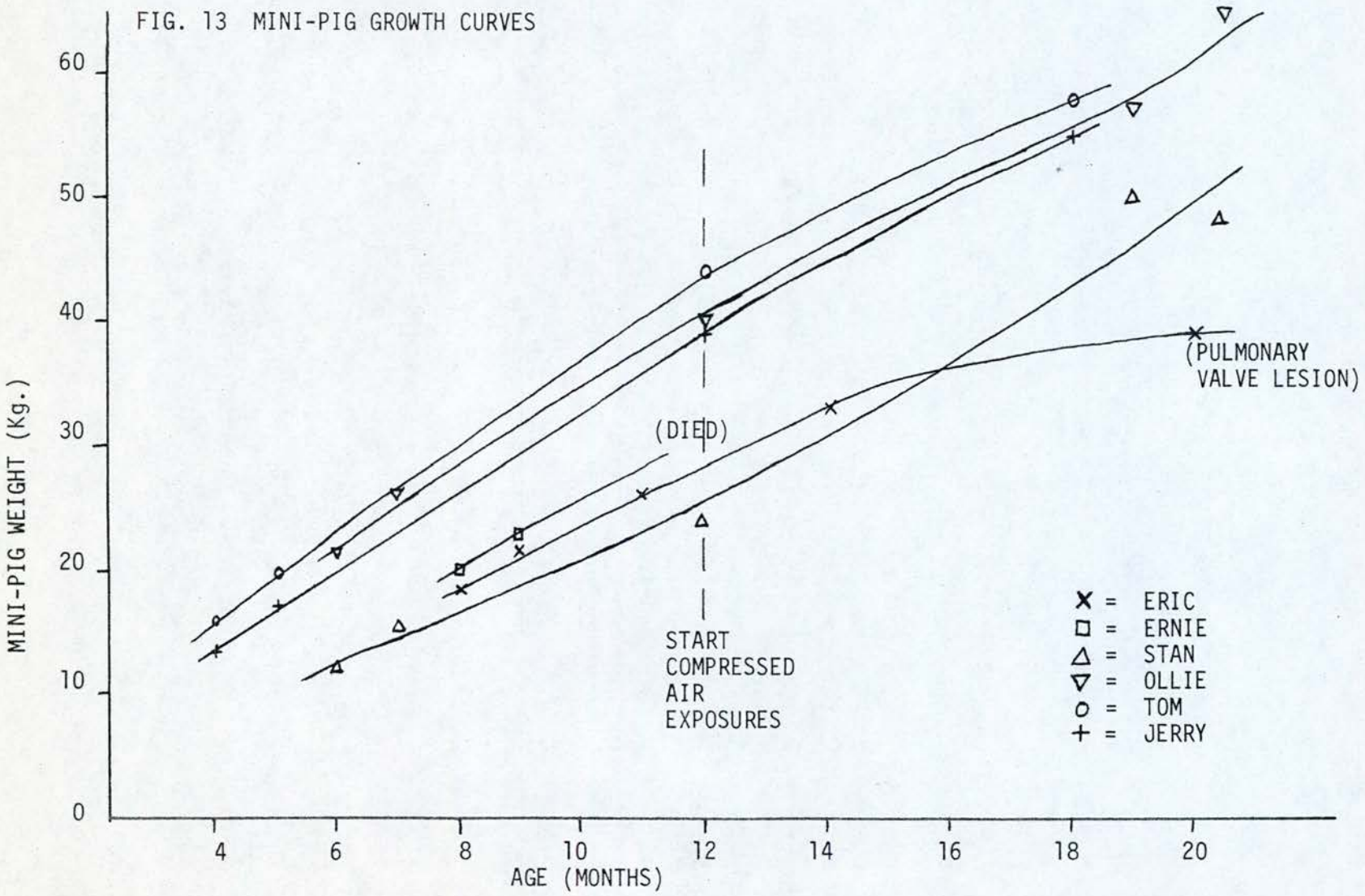
Eric commenced hyperbaric exposure at twelve months of age. After 34 decompressions (188 hours at 27 p.s.i.g.) he collapsed 10 minutes after completion of decompression, turning blue and both hind limbs giving way. This was on a Thursday and subsequent exposure the following Monday was uneventful. This minipig went on to complete 300 hours at 27 p.s.i.g., undergoing 56 decompressions spread over 28 weeks.

Unfortunately, five weeks after starting hyperbaric exposure this minipig developed a wound abcess with signs of systemic toxicity (operation for placement of right atrial catheter - see Experiment 5). Pus from the wound grew a variety of bowel organisms and broad-spectrum antibiotics were administered intravenously for 5 days and the catheter then removed. Eric appeared to make a full recovery from this infection, but post-mortem performed 8 weeks after his last exposure to pressure revealed an endocarditis of the pulmonary valve with a huge vegetative thrombus occluding the pulmonary trunk for a length of over 3 cm. I think this is the probable explanation for the slowing in his weight increase with time compared with the other minipigs (Fig. 13).

Minipig Stan

Stan commenced hyperbaric exposure at twelve months of age. He suffered respiratory bends after 16, 30, 31 and 34 decompressions. Four days after his 35th decompression (having completed 196 hours at 27 p.s.i.g.) he collapsed. He refused food and drink, lay down all the time, had a peripheral cyanosis especially marked on the ears, and respiratory distress with a rapid respiratory rate.

FIG. 13 MINI-PIG GROWTH CURVES



The lung fields sounded fairly clear and he was apyrexial. Veterinary opinion was sought and was to the effect that the slow pulse and fairly clear lung fields (if anything more suggestive of venous congestion than pneumonia) were probably due to myocardial infarction. A four day course of antibiotic was prescribed to prevent any secondary lung infection. It seemed possible that intravascular bubbles might have led to coronary vessel occlusion and Stan was retired from hyperbaric exposure for eight weeks and then cautiously returned to further compressed air experience. After six further exposures (but only two full six hour shifts) a further respiratory bend occurred and after this the shift length was reduced to four hours. Five weeks later a six hour shift was attempted but skin, musculo-skeletal, and respiratory signs developed and recompression was required and appeared to be successful. Four hour shifts were thereafter the maximum used and the minipig finally retired 30 weeks after first hyperbaric exposure having completed 59 decompressions and 282 hours at 27 p.s.i.g.

Stan was the only minipig in the group studied to develop a definite musculo-skeletal bend with lifting of the hind limb off the ground. Respiratory problems at the same time needed recompression and this appeared to successfully treat the affected hind limb also. Post-mortem was performed eight weeks after the last hyperbaric exposure. No abnormality was found; and more specifically, there was no evidence of bone or marrow necrosis when the long bones were sectioned.

Minipig Ollie

Ollie commenced hyperbaric exposure at twelve months of age. He started (together with Stan) with one shift of 4 hours (half shift)

then shifts of 8 hours. After his second complete (8 hour) shift he had an extensive skin bend around his head and neck and rapid respiration only 5 minutes after the end of decompression. After 12 minutes he completely collapsed and was recompressed. After this experience the maximum shift length used for all the minipigs was six hours at 27 p.s.i.g. Despite this, Ollie suffered respiratory bends after his 15th, 33rd, 34th, 46th and 49th decompressions. All responded rapidly to recompression. In addition he had numerous skin bends. An example of a fairly extensive patch of presumed venous stasis is shown in Fig. 14. After his 49th decompression (268 hours at 27 p.s.i.g.) the maximum shift length was reduced to 4 hours and no further recompressions were needed, though extensive skin mottling still occurred. He retired 30 weeks after first hyperbaric exposure having completed 84 decompressions and 393 hours at 27 p.s.i.g.

Post-mortem was performed eight weeks after the last hyperbaric exposure. There were many large air spaces in the medullary cavities of both femora when the bones were sectioned. In addition the cortex of the left humerus appeared thickened and was submitted to microscopic examination but no bone necrosis was found. There was no other macroscopic evidence of bone or marrow necrosis.

Minipig Tom

Tom commenced hyperbaric exposure at thirteen months of age. He suffered from respiratory bends after his 10th, 25th, 44th, 49th and 57th shift. Two of these decompressions (44th and 49th) followed exposures of only 4 hours at 27 p.s.i.g. and the latter of these was a rapid collapse with convulsions 40 minutes after the end of decom-

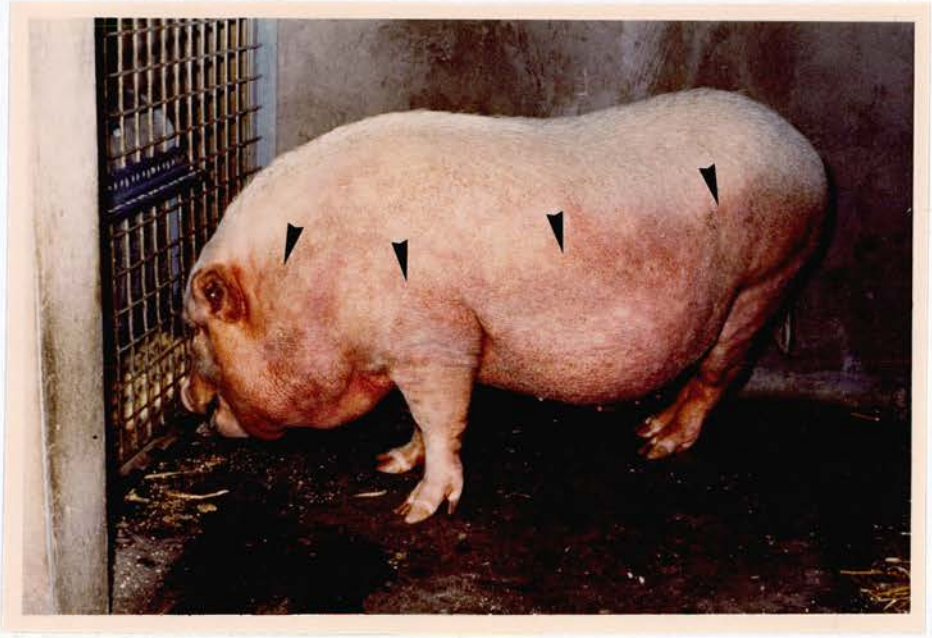


Fig. 14 Minipig Ollie showing extensive area of venous stasis following decompression.

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pression. In addition to these episodes small areas of skin bends were occasionally observed. Tom retired 19 weeks after first hyperbaric exposure, having completed 63 decompressions and 317 hours at 27 p.s.i.g.

Post-mortem was performed six weeks after the last hyperbaric exposure. Several of the long bones showed air spaces within the medullary cavity, but no sign of bone or marrow necrosis.

Minipig Jerry

Jerry commenced hyperbaric exposure at thirteen months of age. He suffered respiratory bends after his 39th, 40th and 41st decompressions, despite a four day interval between his 40th and 41st hyperbaric exposure. The maximum shift length was reduced from 6 hours to 4 hours but further emergency recompressions for respiratory problems were necessary after the 47th and 48th decompressions. Occasional skin bends were also observed. He retired 19 weeks after first hyperbaric exposure, having completed 64 decompressions and 320 hours at 27 p.s.i.g.

Post-mortem was performed six weeks after the last hyperbaric exposure.

All minipigs were killed by overdose of intravenous pentobarbitone (60mg./Kg.) after the induction of general anaesthesia with a nitrous oxide/oxygen/halothane mixture by mask. The long bones of all six minipigs were removed after death, submitted to radiographic examination (Watson MX4 Xray Unit, Ilfex film), then frozen. Both femora, humeri and tibiae were treated in this way and once frozen they were longitudinally sectioned on a heavy-duty water-cooled rotary

diamond saw (A. and D. Hughes Ltd., Oldbury, Worcs.) to observe any macroscopic lesion. Care was taken to section the middle of the femoral and humeral heads. No lesion suggestive of bone or marrow necrosis was found on radiographic or macroscopic examination. Two animals (Tom and Ollie) showed extensive air spaces in the medullary cavities of several long bones (Fig. 15), presumably equivalent to the "aeroembolism" reports noted in Table 2. This appearance was not seen in the two control animals of similar age studied in conjunction with Gregg (see Gregg, 1977).

No attempt was made to perform a comprehensive histological examination on these bones, although this appears to have been done by Stegall and Smith (1974) and Wünsche and Scheele (1973). There were two reasons for this. The first is that any lesion only found on microscopic examination would obviously not be comparable to the lesions of caisson disease of bone seen in affected human beings and it might be questioned whether it was the same disease process. The second reason is that looking through the microscope trabeculae can appear devoid of osteocytes except for appositional new bone on the surface even in control specimens. Figure 16 shows a 6µm section from the control femur (left side) of a rabbit in Experiment 7. It might be claimed to show osteonecrosis with appositional new bone formation. It is x320 magnification and greater than the magnification usually used in published accounts of osteonecrosis found in animals after hyperbaric exposure.

Gregg (1977) compromised by selecting one bone from each minipig for histological examination, but he found no evidence of bone or marrow necrosis except in relation to a lesion which was obvious macroscopically.

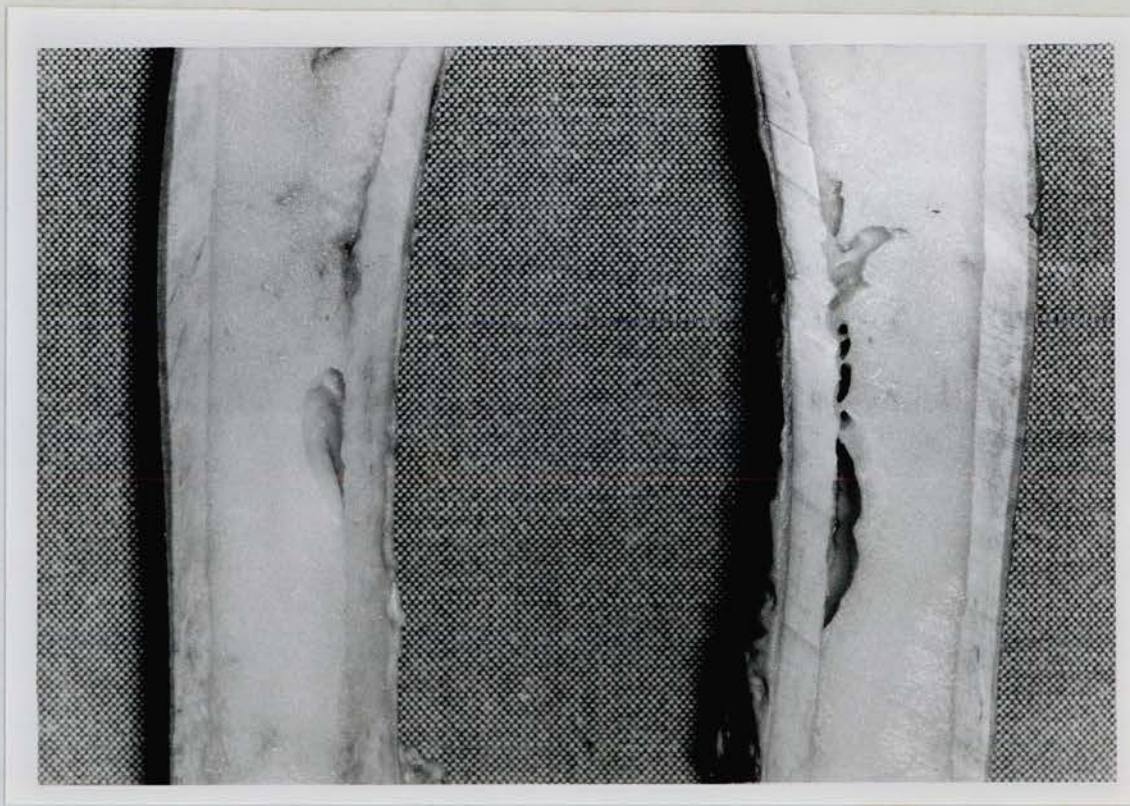


Fig. 15 "Aeroembolism" of bone marrow. Example shown is a femur of minipig Tom.

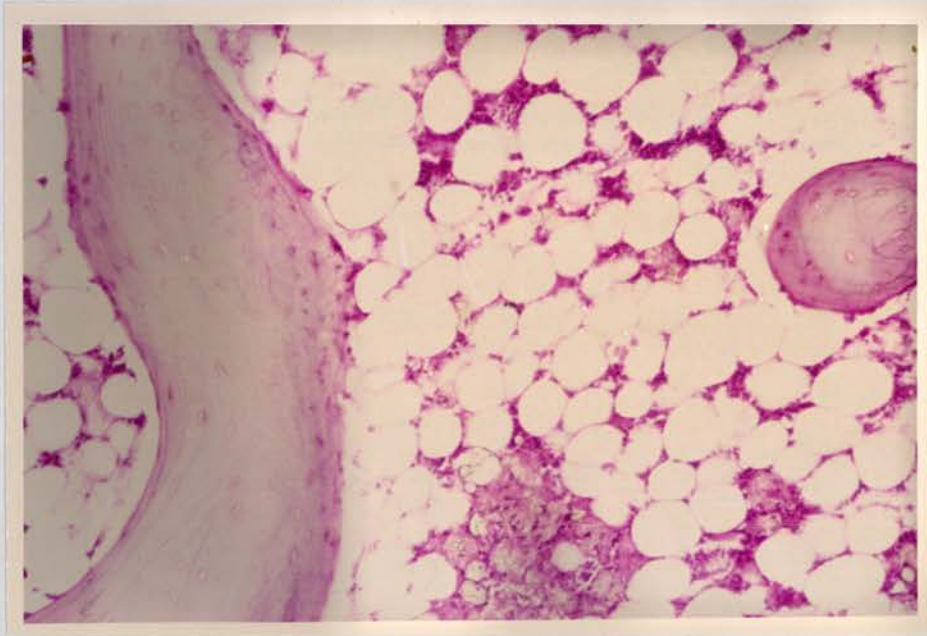


Fig. 16. Carefully selected area from control rabbit femur. The lacunae in the central part of the trabeculae appear devoid of osteocytes (see text). x 320.

Regions with suspected abnormalities on macroscopic examination or skeletal scintigraphy or on radiography (Experiment 6) were submitted to routine histological examination. Sections were fixed in 10% formol saline, decalcified, embedded in paraffin, sectioned on a Reichert sledge microtone, and suitable sections about 8 μm thick mounted and stained with Harris's Haematoxylin and Eosin. No bone or marrow necrosis was found on microscopic examination of sections from areas suspected to be abnormal.

Discussion

The compression, exposure, and decompression profile used in this experiment produced a substantial number of episodes of symptomatic acute decompression sickness which responded to therapeutic recompression. It has been well documentated that compressed air workers become less susceptible to acute symptoms with repeated exposures i.e. they 'acclimatise'. This was first described by Paton and Walder (1954). This behaviour was not exhibited by the miniature pigs. The graphs of their exposure times (Fig. 11) and decompressions (Fig. 12) show that the pigs had more acute decompression sickness after they had completed a substantial number of exposures. This has been previously noticed by Smith and Stegall (1974), who record that "It was not intended that the exposures be fatal. Nonetheless several animals were lost to acute decompression sickness after 35 to 40 asymptomatic exposures." In addition to this we noticed that some of the minipigs seemed to become more susceptible to attacks of acute decompression sickness after this and would develop signs even after the first decompression of a week. The first episode of decompression sickness of each minipig was always after

three or four shifts on consecutive days (Fig. 11). However, once an animal became susceptible to acute decompression sickness it was sometimes affected on the first decompression after the weekend break, that is the minipigs appeared to "deacclimatise". As each animal automatically had a day without hyperbaric exposure following a therapeutic recompression this produced a substantial number of episodes of acute decompression sickness recorded as following shift 1. Table 6 shows the overall pattern of attacks of acute decompression sickness requiring therapeutic recompression. There is a noticeable freedom from symptoms after shift 2.

Studies of compressed air contracts have concentrated on acclimatisation after long breaks without hyperbaric exposure but one report reviewed the incidence of acute decompression sickness on each day of the week to see if there was an increased susceptibility following pay-day. Pay-day was Thursday and the only day with a significantly higher "bends rate" than other days was Sunday, when only watchmen were exposed. (Singstad, 1944). The most likely explanation may well be the absence on Sundays of non-shift workers only being exposed to pressure for short intervals but adding to the recorded number of man-decompressions on the other days.

Conclusions.

The compression/decompression profile used did not lead to the development of caisson disease of bone in 6 Göttingen miniature pigs. The pigs were castrated males subjected to repeated hyperbaric exposures (range 35 to 84) commencing at 12 months of age. The exposure profile was the same as that successfully used by Gregg

(1977).

Aeroembolism of the bone marrow was found at autopsy in 2 of the 6 minipigs.

EXPERIMENT 4. ESTIMATION OF URINARY HYDROXYPROLINE EXCRETION IN GOTTINGEN MINIATURE PIGS UNDERGOING REPEATED EXPOSURE TO EXCESS PRESSURE.

HYPOTHESIS.

Since the suggestion by Deiss (1974) that an early indication of an area of osteonecrosis might be a detectable increase in the urinary hydroxyproline excretion there have been relatively few attempts to perform such measurements following decompression and none in subjects who are known to have developed osteonecrosis. The few results available are discussed in Chapter 3.

Because of the successes claimed at producing dysbaric osteonecrosis in miniature swine (Smith and Stegall, 1974; Gregg, 1977), it was decided that it would be interesting to measure urinary hydroxyproline excretion in the group of miniature swine being studied in Experiment 3. Weatherley (1976) had already attempted to measure 24 hour total urinary hydroxyproline excretion levels in miniature swine without osteonecrosis and found wide day to day fluctuations. He had placed each minipig in a metabolic cage for the period of urine collection and it was thought that this change of pen might have influenced the urine output. The miniature swine had often been observed to micturate only at intervals in excess of 24 hours, causing inaccuracy in measurements of urine constituents corrected to a 24 hour urine volume. It was evident that precautions would have to be taken in the design of the experiment to try and achieve reliable results and before the start of the experiment two major decisions were taken. One was not to move the minipig from its usual cage for the days when measurements were required and the other was to

only use values based on measurements of urinary hydroxyproline excretion over a period of three consecutive 24 hours.

MATERIALS AND METHODS.

The miniature swine used and the exposures to excess pressure are detailed in Experiment 3.

The method of making collections of 24 hour urine samples was to erect two false floors in the minipigs' normal pens. One was a sturdy solid galvanised trough sloping to a narrow waste pipe welded to a hole at one end. This was supported on four concrete blocks. The waste pipe was positioned beneath a non-spill water trough bolted to the pen door. This arrangement was entirely satisfactory. The waste pipe was only $\frac{1}{2}$ inch diameter and gross contamination of the collected urine with faecal material was at an acceptably low level. The other false floor was made of perforated aluminium sheet supported on a frame of Dexion brand angle iron. The urine was collected along a sloping double thickness polythene sheet into a two gallon plastic bucket. This perforated sheet trapped almost all the pigs' faecal material. Occasionally one minipig (Jerry) was able to tear the aluminium sheet off the supporting Dexion frame with his teeth, ripping the securing wires through the perforations, thus ruining the urine collection by allowing gross faecal contamination.

The non-spilling water troughs were of standard design and were made from galvanised steel sheet by Boyd and Company Ltd., Chain Bridge Road, Blaydon-on-Tyne. Each trough was bolted to the pen door so that it could easily be refilled. The water intake of the minipigs was not restricted.

At least eight days before starting exposure to pressure, each minipig was placed in one of the pens with a raised floor, and it remained in this pen until measurements were completed except during time spent in the compression chamber. In most weeks the minipigs were exposed to pressure for four consecutive days (usually Monday to Thursday) and 24 hour urine collections were performed on the other days (usually Friday, Saturday and Sunday).

The samples from the seven days before the minipigs started their exposures to pressure served as a baseline and emphasised the fluctuations in day-to-day urine volumes and hydroxyproline excretion. Minipigs micturate infrequently (only once or twice in each 24 hours) and certainly not at 8 o'clock each morning. This means that measurements based on single 24 hour specimens would be inaccurate. It was hoped to reduce this by measuring the values for three consecutive days and determining the mean value. This could be compared with the mean value of the pre-exposure measurements. A further precaution was to recheck these baseline values at least six weeks after the minipig had completed its series of exposures to pressure. This seemed necessary because the animals were growing and the increased size and weight might have affected the values. However, it should be noted that the rate of increase in weight did not exceed that at twelve months of age when the baseline was measured and the minipigs started their exposures to pressure (see Fig. 13).

The urine volumes were measured in a litre measuring cylinder to the nearest 5 ml. 20 ml. aliquots were placed in clean tubes, labelled, and stored at $-20^{\circ}C$. until assayed. No preservative was added.

Total urinary hydroxyproline was estimated by the method of

Goverde and Veenkamp (1972). This assay has been used by the laboratory for several years and found to give reproducible results when tested by duplicate estimations.

Each minipig was sacrificed at least six weeks after it had completed its series of exposures to pressure and the femora, humeri, and tibiae were examined as described in Experiment 3.

RESULTS.

The measurements of daily 24 hour urinary volume and total urinary hydroxyproline are shown in Table 7, together with the mean values of each set of samples amounting to at least three consecutive days' results. Fig. 17 shows a histogram of the baseline values (before exposure to pressure) of the 24 hour total hydroxyproline excretion. It can be seen that these values do not confirm to a normal distribution curve. The high values probably represent days when the minipigs passed urine also including the previous day's excretion of hydroxyproline. Fig. 18 shows a histogram of the 3 day values taken at the beginning and end of the pre-exposure measurements. There are only twelve values but the variation is less and using these values gives a mean value of 26.6 $\pm$ 10.5 mg total urinary hydroxyproline per 24 hours compared with a mean value of 27.5 $\pm$ 14.2 mg / 24 hours for the figures based on the single 24 hour collections for the same days.

The baseline values were rechecked on four of the six minipigs six weeks after their last hyperbaric exposures (Table 8). The values were all increased. I am advised that it is not valid to

TABLE 7. MEASUREMENTS OF INDICES OF COLLAGEN BREAKDOWN (Serum Proline Imino Peptidase and 24h total urinary hydroxyproline excretion) in MINIATURE SWINE UNDERGOING REPEATED HYPERBARIC EXPOSURE.
Serum PIP in mU/ml/min Urine Volume in ml. Urine Hypro. in mg./24h

ERIC. D = Hyperbaric Exposure.

DATE	SERUM PIP	URINE VOLUME	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOLUME	URINE HYPRO.	MEAN
8/3		1470	27.5		6/4	0.14	D		
9		1350	19.9	26.6	7	0.35	D		
10	0.78	800	32.4		8	0.22	1250	10.5	
11	0.47	600	14.3		9	0.25	1275	14.8	
12	0.25	740	30.4		10	0.21	1040	10.0	11.6
13	0.12	960	30.2	27.6	11		650	11.3	
14	0.13	1000	22.2		12		800	11.4	
15		D			13		D		
16	0.11	D			14		D		
17	0.34	D			15	0.29	D		
18		D			16	0.20	370	21.1	
19	0.73	650+	10.7+		17		470	40.5	34.5
20		650+	8.0+	9.5	18		850	41.9	
21		800+	9.9+		19	1.75	D		
22		D			20		D		
23		D			21		D		
24		D			22		Xray scan		
25		D			23		675D		
26		800	8.6		24		550	27.3	
27		600	10.1	10.2	25	0.30	600	35.7	
28	0.14	200+	11.9		26		550D	56.9	45.3
29	0.13	D			27		750	61.8	
30	0.17	D			28		D		
31	0.09	D			29		D		
1/4	0.33	D			30		650	31.9	
2	0.19	765	13.1		1/5		550	39.7	37.9
3	0.14	970	12.9	13.1	2		500	42.1	
4	0.21	1060	13.4		3		D		
5	0.18	D			4		D		

TABLE 7 continued ERIC continued

DATE	SERUM PIP	URINE VOLUME	URINE HYPRO.	MEAN
4/5		D		
5		D		
6		D		
7		D		
8				
9				
10		D		
11		D		
12		D		
13		D		
14				
15				
16				
17		750D		
18		850		
19		700D		
20		950D		
21		500+D		
22		700	43.3	
23		950	69.8	52.7
24		350	45.0	
25				
26				
27		D		

ERNIE

TABLE 7 continued D = Hyperbaric Exposure

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
8/3		1750	12.0		6/4	0.22	D		
9		2300	7.9	10.4	7	0.40	D		
10	0.85	950	11.4		8	0.20	1370	21.1	
11	0.27	500	7.6		9	0.30	950	25.4	
12	0.15	90	5.8		10	0.15	380	16.9	20.2
13	0.08	550	18.3	13.8	11	0.22	630	21.1	
14	0.24	750	17.4		12	0.42	650	16.3	
15		D			13	0.42	D		
16	0.23	D			14	0.26	D		
17	0.19	D			15	0.35	D		
18	0.23	D			16	0.35	1450	20.1	
19	0.30	1950	65.7		17	0.59	780	20.8	28.7
20	0.37	1150	19.9	37.1	18	0.18	1500	45.3	
21	0.19	900	25.8		19	0.29	D		
22	0.21	D			20	0.32	D		
23	0.67	D			21	0.34	D		
24	0.17	D			22	0:24	Xray scan		
25	0.19	D			23		D		
26	0.06	2250	53.7		24		1100	-	
27	0.22	550	24.5	28.4	25				
28	0.17	100	6.9		26		D		
29	0.30	D			27		1000		
30		D			28		D		
31	0.20	D			29		D		
1/4	0.45	D			30		850	22.8	
2	0.25	1000 D	29.5		1/5		900	13.8	15.2
3	0.32	750	15.8	28.5	2		325	9.1	
4	0.32	1000	40.2		3		D		
5	0.26	D			4		D		

ERNIE continued

TABLE 7 continued

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
4/5		D		
5		D		
6		D		
7		D		
8				
9				
10		D		
11		D		
12		D		
13		D		
14		250	4.8	
15		250	12.5	15.2
16		650	28.3	
17				
18		D		
19				
20				
21				
22				
23				
24		150	7.9	
25		400	25.9	17.7
26		400	18.6	
27		475	18.4	

TABLE 7 continued D = Hyperbaric Exposure

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
4/5		1900	33.7		14/6		D		
5		1650	26.9	32.3	15		D		
6		1800	36.3		16		D		
7		870	18.5		17		D		
8		1200	33.0		18		2000	55.1	
9		525	15.6	24.8	19		1300	41.5	46.7
10		700	25.7		20		1400	43.6	
					21		D		
24		D			22		1250	40.0	
25		D			23		1200	22.9	
26		D			24		D		
27					25		950D	45.0	
28		660D	27.5		26		1000	62.8	50.1
29		650	21.4	28.6	27		1000	42.4	
30		725	36.9		28	0.49	D		
31		D			29	0.30	D		
1/6		D			30	0.22	D		
2		D			1/7	0.28	D		
3		D			2	0.23	1000D	52.7	
4		750	28.3		3	0.28	780	45.5	44.7
5		650	41.4		4	0.24	700	36.0	
6		760	25.8	25.8	5	0.20	D		
7		700	24.1		6	0.17	D		
8		200	9.4		7	0.23	D		
9		D			8	0.25			
10		D			9	0.41	625	12.9	
11		1150D	51.7		10	0.40	1200	59.3	34.3
12		1200	54.5	46.3	11	0.28	500	30.7	
13		1000	32.6		12	0.25	D		

STAN continued

TABLE 7 contd D = Hyperbaric Exposure

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
13/7	0.35	D			30/1		1350	28.9	
14	0.34	D			1/2		600	32.1	
15	0.31	D			2		800	24.7	
16		900	47.8		3		1150	38.6	31.6
17	0.29	1200	33.1	38.8	4		1300	30.2	±
18	0.25	1000	35.5		5		500	35.7	4.5
19	0.15	D			6		500	31.3	
20	0.46	1100	61.3						
21	0.16	D							
22	0.32								
23	0.33	1350	26.8						
24	0.34	1200	37.2						
25	0.27	1300	-						
26	0.26	850	33.4						
27	0.20								
28	0.38								
29	0.51								
30	0.25								
31	0.31								
1/8	0.40								
2	0.32	D							
3	0.37	D							
4		D							
5									
6									
7		800	24.6						
8		1035	39.0						
9		900	-						

TABLE 7 CONTD. D = Hyperbaric Exposure

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
4/5		250	13.2		14/6		D		
5		1900	lost	19.9	15		D		
6		1100	17.4		16		D		
7		700	29.0		17		D		
8		340	18.7		18		575	32.1	
9		900	41.9	25.6	19		1350	59.4	42.6
10		450	16.1		20		700	36.3	
					21		D		
24		D			22		1300	62.9	
25		D			23		1250	48.6	
26		D			24		D		
27					25		700D	38.0	
28		700	35.5		26		900D	58.0	43.1
29		730	29.2	24.4	27		700	33.3	
30		300	8.7		28	0.51	D		
31		D			29	0.09	D		
1/6		D			30	0.09	D		
2		D			1/7	0.12	D		
3		D			2	0.17	950D	62.3	
4		600	16.7		3	0.08	650	-	
5		400	18.3		4	0.08	500	23.2	
6		650	46.0	32.7	5	0.06	D		
7		1100	46.3		6	0.09	D		
8		1100	36.0		7	0.09	D		
9		D			8	0.21			
10		D			9	0.48	700	36.9	
11		750D	27.7		10	0.44	600	24.2	28.9
12		1350	54.9	44.9	11	0.42	490	25.5	
13		1000	52.0		12	0.39	D		

TABLE 7 CONTD. OLLIE continued

D = Hyperbaric Exposure

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
13/7	0.44	900D	40.3		7/2		550	35.9	
14	0.42	900D	52.6	45.6	8		1200	36.2	
15	0.32	1200D	43.9		9		1150	43.1	36.8
16	0.59				10		1100	42.8	±
17	0.49				11		1600	50.0	
18	0.31				12		300	12.5	11.9
19	0.14	D			13		300	37.0	
20	0.16	1100	60.9						
21	0.24	D							
22	0.16								
23	0.27	1300D	48.8						
24	0.34	1450	56.6	45.0					
25	0.38	900	29.6						
26	0.22	D							
27	0.11								
28	0.29	D							
29	0.22								
30	0.25								
31	0.29								
1/8									
2		D							
3		D							
4		D							
5		D							
6									
7		400	16.2						
8		850	33.6	20.1					
9		300	10.5						

TOM

TABLE 7 contd.

D = Hyperbaric Exposure

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
27/7		450	25.0		25/8	0.43	D		
28	0.44	550	23.0	30.0	26	0.32	D		
29		600	41.9		27		400	106.7	
30		750	40.0		28		1250	92.2	74.9
31		510	24.0		29		900	72.8	
1/8		830	46.1		30		1100	28.0	
2		1700	44.5		31	0.21	D		
3		1290	39.3		1/9	0.37	D		
4		1130	21.6		2		D		
5		500	20.8	19.1	3	0.33	1010	70.8	
6		1400	14.9		4	0.29	1440	67.0	69.5
7					5	0.27	1250	70.6	
8					6	0.28	D		
9		D			7	0.25			
10		D			8	0.35	D		
11		D			9	0.42	D		
12		D			10	0.39	740	41.5	
13		1950	63.4		11		950	65.9	51.4
14		900	71.5	63.1	12	0.44	450	46.7	
15		650	54.6		13	0.62	D		
16		D			14	0.39	D		
17	0.39	D			15	0.39	D		
18	0.28				16		D		
19		D			17	0.33	1200 D	65.2	
20	0.24	950	62.1		18	0.59	1300	80.4	
21		1000	75.0	63.2	19	0.30	lost	-	
22	0.27	370	28.8		20	0.34	D		
23	0.26	D			21	0.29	D		
24	0.31				22	0.37	D		

TOM continued

TABLE 7 contd. D = Hyperbaric Exposure.

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
23/9		D			22/10	0.13	2750	61.9	
24	0.68	1700	117.6		23		850	43.8	42.2
25		1250	76.1	77.3	24	0.20	150	21.0	
26	0.44	450	38.2		25	0.44	D		
27	0.42	D			26	0.28			
28	0.50	D			27	0.19	D		
29	0.54				28	0.42	D		
30	0.30	D			29	0.25	lost	-	
1/10	0.33	900	34.7		30	0.26	1250	67.7	
2	0.47	700	49.8	49.7	31	0.27	750	61.0	
3		1100	64.6		1/11	0.27	D		
4		D			2	0.20	D		
5		D			3	0.27	D		
6	0.43	D			4	0.47	D		
7	0.30	D			5	0.25	1000 D	39.1	
8	0.38	800	46.5		6	0.25	600	46.9	43.5
9		1000	67.5	48.3	7	0.31	400	35.7	
10	0.26	700	30.9		8	0.26	900	52.4	
11	0.14	D			9	0.26	D		
12	0.29	D			10	0.27	1100	45.4	
13		D			11		1600	57.5	
14		D			12		2450	45.4	47.9
15		1360D	62.8		13		410	35.6	
16		1300	46.3	55.4	14		1200	55.8	
17	0.26	1350	57.1		15		D		
18	0.27	D			16		D		
19	0.29	D			17		D		
20	0.15				18		D		
21		D			19		2500	62.7	

TOM continued

TABLE 7 contd. D = Hyperbaric Exposure

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
20/11		1750	50.7	53.9	26/1		1150	40.7	
21		750	48.2		27		1400	44.3	39.0 ± 6.7
22		D			28		1050	45.9	
23		D			29		2620	30.4	
24		D			30		2600	33.7	
25									
26		1700D	61.4						
27		1000	46.1	52.8					
28		750	51.0						
29		D							
30		1700	83.5						
1/12		850	46.8						
2		1250	49.0	58.0					
3		1900	57.0						
4		1200	53.5						
5									
6		D							
7		D							
8		D							
9									
10									
11									
12									
13		2300D	63.0						
14		950D	52.4	52.1					
15		950D	50.3						
16		1100D	42.8						

JERRY

TABLE 7 contd.

D = Hyperbaric Exposure.

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
27/7		900	42.8		25/8	0.37	D		
28	0.62	1100	47.7	41.6	26	0.42	D		
29		0	0		27	0.73	1200	20.0	
30		930	76.1		28	0.86	570	46.4	40.9
31		900	52.7		29	0.47	625	50.0	
1/8		0	0		30	0.50	600	47.3	
2		760	35.3		31	0.49	D		
3		620	32.6		1/9	0.78	D		
4		1420	83.7	47.1	2	0.70	D		
5		555	21.5		3	0.72	850	42.8	
6		800	50.7		4		1000	57.4	57.4
7					5		1300	72.1	
8					6		D		
9		D			7	0.41			
10		D			8		D		
11		D			9		D		
12		D			10	0.67	540	20.9	
13		600	29.1		11		520	36.6	34.0
14		545	29.3	32.1	12	0.43	600	44.4	
15		700	38.0		13	0.31	D		
16		D			14	0.26	D		
17	0.62	D			15	0.28	D		
18	0.49				16		D		
19	0.34	D			17		350+D	15.9	
20	0.30	0	0		18	0.56	1500	50.4	
21		1700	lost		19	0.40	lost	-	
22	0.31	1100	59.6		20	0.44	D		
23	0.45	D			21	0.52	D		
24	0.27				22	0.29	D		

JERRY continued

TABLE 7 contd. D = Hyperbaric Exposure

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
23/9		D			22/10	0.53	550	20.0	
24	0.48	1000	57.9		23		0	0	
25		1050	56.3	46.4	24	0.52	0	0	
26		400	25.0		25	0.32	D		
27	0.76	D			26	0.41			
28	0.74	D			27	0.46	D		
29	0.31				28	0.40	D		
30	0.42	D			29	0.52	150	5.5	
1/10	0.45	500	33.0		30	0.60	500	35.2	16.0
2	0.47	550	41.1	41.4	31	0.51	150	7.2	
3	0.60	700	50.1		1/11	0.42	D		
4		D			2	0.51	D		
5	1.02	D			3	0.46	D		
6	0.78	D			4	0.42	D		
7	0.49	D			5	0.35	1700	101.6	
8	0.77	1200	75.5		6	0.44	750	50.0	54.8
9		900	48.6		7	0.42	400	25.4	
10	0.61	-	-		8	0.36	770	42.3	
11	0.83	D			9	0.46	D		
12	0.53	D			10	0.52	700	32.7	
13	0.56	D			11		100	8.0	
14	0.16	D			12		700D	37.8	24.0
15	0.55	840D	30.3		13		550	34.7	
16	0.40	1340	28.9	33.7	14		700	7.0	
17	0.45	1200	41.9		15		D		
18	0.30	D			16		D		
19	0.33	D			17		D		
20	0.40				18		D		
21	0.51	D			19		750	35.4	

JERRY continued

TABLE 7 contd.

D = Hyperbaric Exposure

DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN	DATE	SERUM PIP	URINE VOL.	URINE HYPRO.	MEAN
20/11		1250	47.1	46.9					
21		550	58.1						
22		D							
23		D							
24		D							
25		D							
26		D							
27		—							
28		—							
29		D							
30									
1/12									
21/1		650	41.4	44.0 ± 38.1					
22		650	41.6						
23		0	0						
24		1100	93.1						
25		0	0						

FIG. 17 DISTRIBUTION OF PRE-EXPOSURE BASELINE VALUES OF 24 HOUR TOTAL URINARY HYDROXYPROLINE EXCRETION (6 MINIPIGS, AGE 12 MONTHS, n = 47)

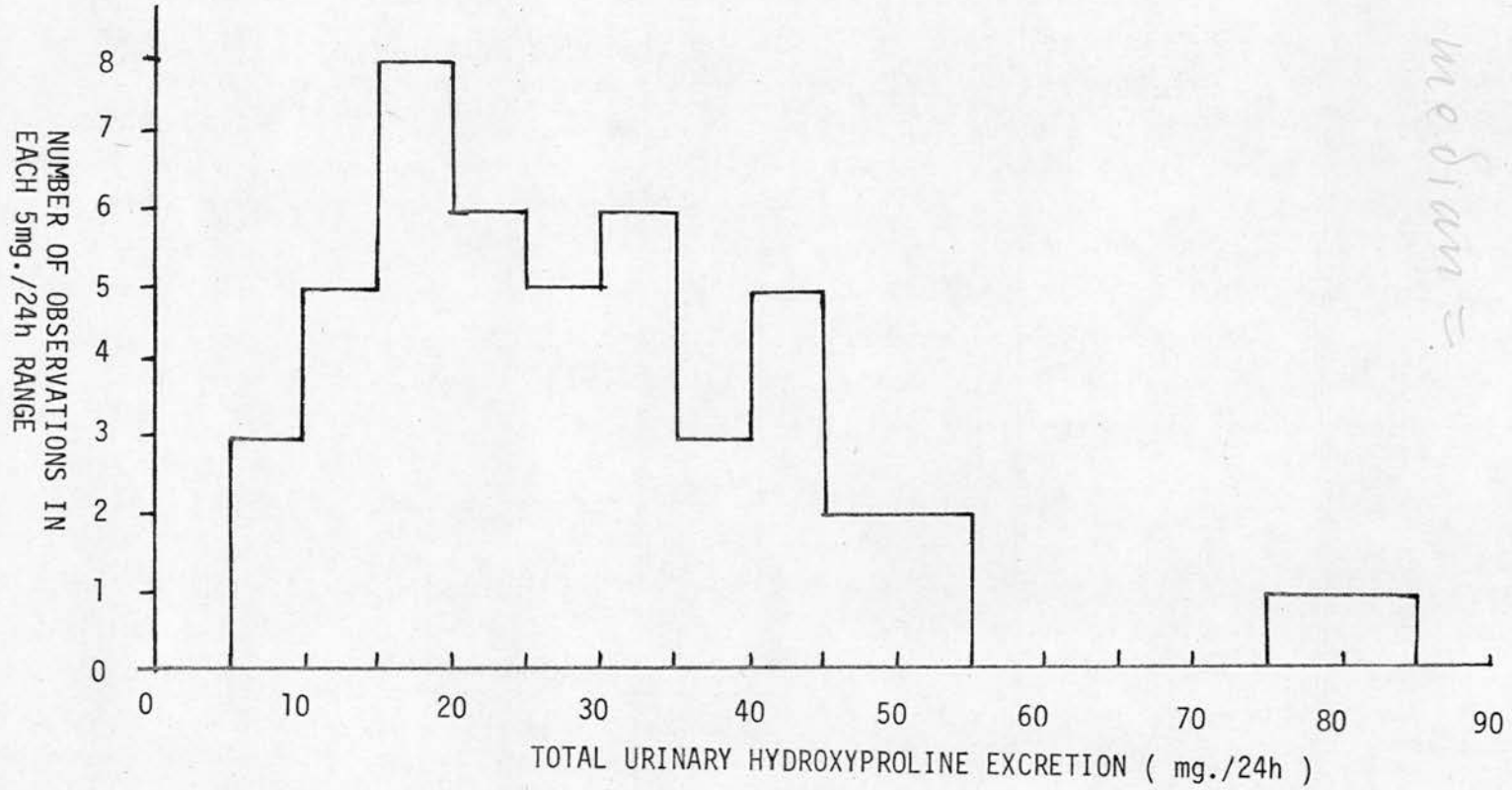


FIG. 18 DISTRIBUTION OF PRE-EXPOSURE BASELINE VALUES OF THREE DAY AVERAGES OF 24 HOUR TOTAL URINARY HYDROXYPROLINE EXCRETION (SEE TEXT) (6 MINIPIGS, AGE 12 MONTHS, n = 12)

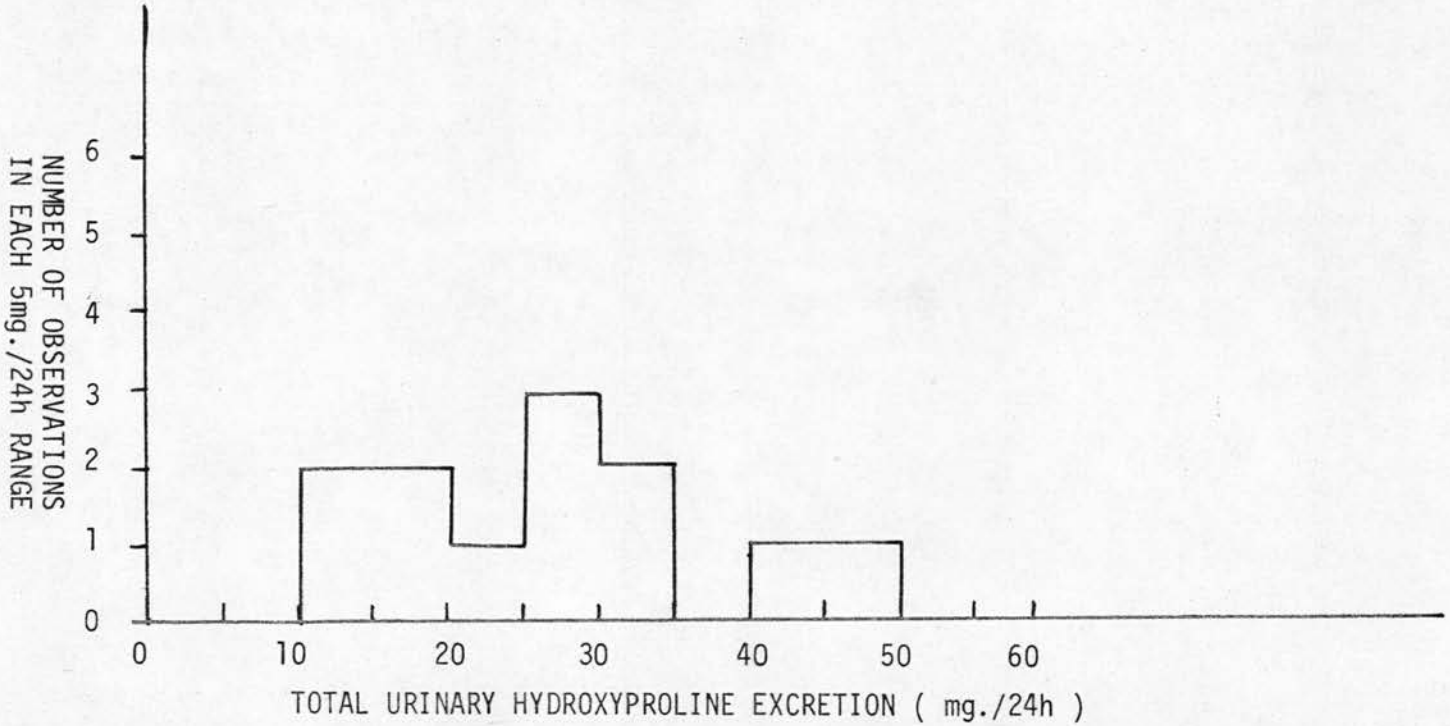
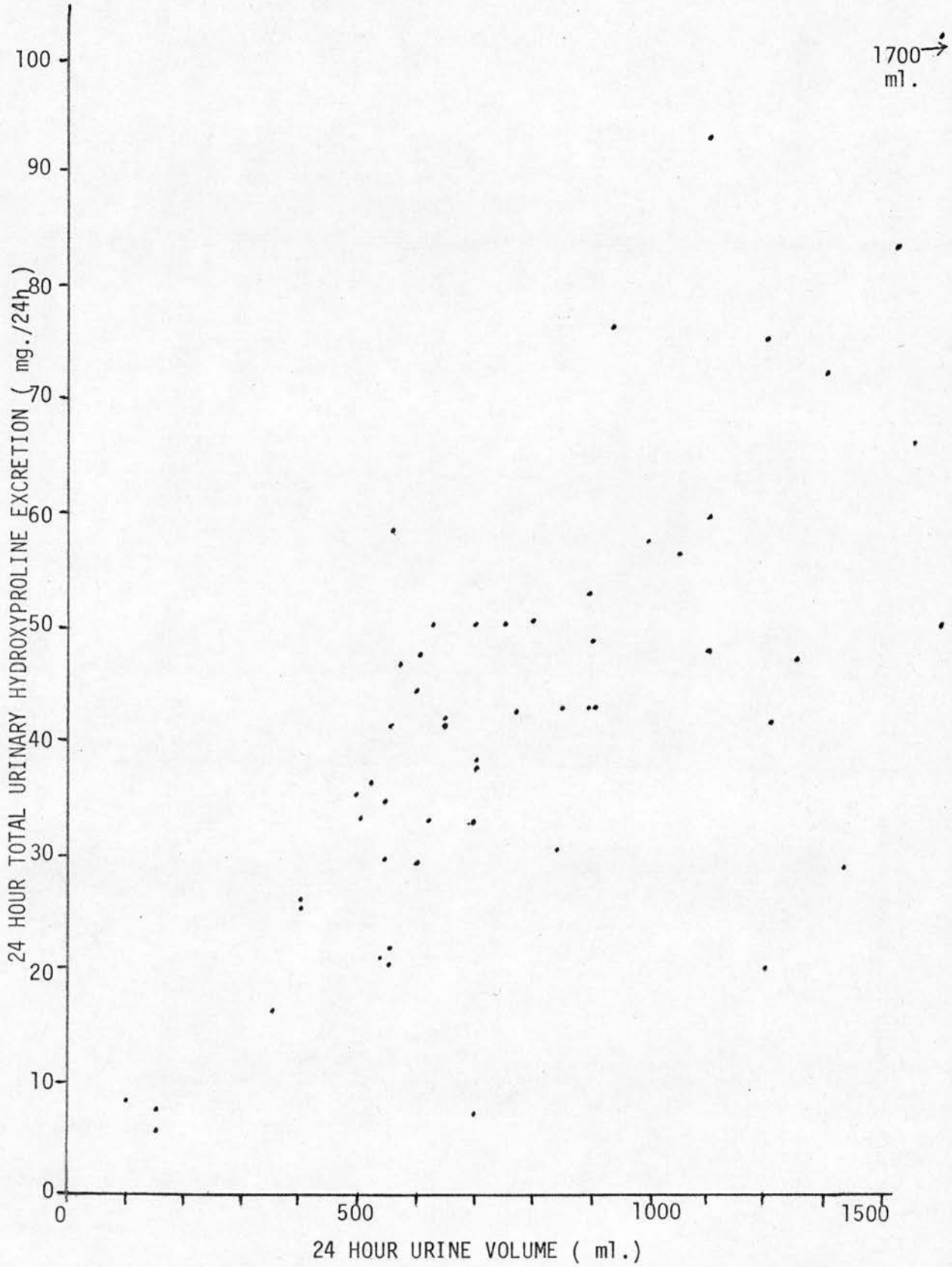


TABLE 8. COMPARISON OF PRE-EXPOSURE AND POST-EXPOSURE BASELINE VALUES OF TOTAL URINARY HYDROXYPROLINE. (SINGLE 24 hour MEASUREMENTS).

MINIPIG	PRE-EXPOSURE			POST-EXPOSURE		
	n	mean	S.D.	n	mean	S.D.
ERIC	7	25.3	6.7	-	-	-
ERNIE	7	11.5	4.9	-	-	-
STAN	7	27.1	7.8	7	31.6	4.5
OLLIE	6	22.7	10.8	7	36.8	11.9
TOM	11	31.0	11.3	5	39.0	6.7
JERRY	11	40.3	26.8	4	44.0	38.1

FIG. 19 CORRELATION BETWEEN 24 HOUR URINE VOLUME AND 24 HOUR TOTAL URINARY HYDROXYPROLINE EXCRETION (MINIPIG JERRY)



statistically compare these figures because they are not an independant variable. The reason for this is shown in Fig. 19, which demonstrates a correlation between the 24 hour total urinary hydroxyproline excretion and the 24 hour urine volume. This was most obvious for minipig Jerry (which showed the widest variations of urine volume) but there also appeared on inspection to be a correlation for the other minipigs. The reason for this correlation is that the urine collections are not true 24 hour collections, as has already been mentioned.

This is important as it would lead to false positive results showing greater hydroxyproline excretion than normal if the minipigs had a diuresis in the days when they were not exposed to pressure.

The results in Table 7 show no sign of a consistent diuresis in the days following hyperbaric exposure in any of the experimental animals. The values of urinary hydroxyproline show substantial variation with some values greater and some less than the baseline mean values. If the baseline value for the averaged 3 day collections is regarded as representing values in a normal distribution curve, then the values in minipig Tom are consistently outside the 2 S.D. range of 5 - 48 mg / 24h. None of the other minipigs had more than one value outside this range. Minipig Tom did not show any bone necrosis on post-mortem examination as described in Experiment 3.

DISCUSSION.

The main inherent error in this experiment is the inaccuracy of the 24 hour urine collections because of the minipigs infrequent voiding. Sometimes there was no urine voided for a full 24 hour period. The obvious way to overcome this problem would have been

to have indwelling catheters in the minipigs. This was deliberately not done because of the likelihood of infection at a time when the animals had indwelling right heart cannulae as part of other experiments (one of which is reported here - Experiment 5.)

Having accepted this limitation of accuracy, it still seemed possible to work out a normal range and compare the mean values of three 24 hour collections with this. None of the 6 minipigs developed bone necrosis and all these values would therefore be expected to fall within the normal range.

This was not the case for minipig Tom. As soon as his hyperbaric exposures had started the urinary hydroxyproline excretion rose markedly and remained elevated throughout the period of hyperbaric exposures. As none of the other minipigs had shown any alteration with hyperbaric exposure we expected that Tom might have developed an area of bone necrosis. This was not confirmed upon post-mortem examination but at which only both femora, humeri and tibiae were examined. The reason for increased hydroxyproline excretion in this minipig remains obscure.

I should perhaps here note that the minipigs had a standard day/night cycle, the lighting in the animal house being entirely artificial and controlled by a timeswitch. They might therefore be expected to show a similar diurnal variation of urinary hydroxyproline excretion to that found in man (Mautalen, 1970) so that expression of the hydroxyproline excretion as mg/l rather than mg/24h was thought not to be an allowable approximation to attempt to overcome the inaccuracy of the 24 hour collections.

It is interesting that the hyperbaric exposure does not appear to result in any alteration in urinary hydroxyproline excretion. The only recorded observation of any alteration is that of Heyder and

Tappan (1973b) in human divers, though this was not confirmed in further experiments (Heyder and Tappan, 1974). This is important as it would make urinary hydroxyproline excretion unsuitable as an early indicator of dysbarism-related osteonecrosis.

CONCLUSIONS.

The mean total urinary hydroxyproline excretion of 6 castrated male Göttingen miniature swine aged twelve months is 27.5 $\pm$ 14.2 mg/24h.

The inaccuracies inherent in attempting 24 hour urine collections in uncatheterised large animals make any abnormal measurements difficult to interpret.

There is no evidence of an increase in total urinary hydroxyproline excretion in 5 out of 6 minipigs following repeated hyperbaric exposure using standard compressed air work tables for decompression.

No conclusions can be made about the suitability of total urinary hydroxyproline excretion abnormalities as an early indicator of bone necrosis as none of the experimental animals developed bone necrosis.

EXPERIMENT 5. ESTIMATION OF SERUM PROLINE IMINOPEPTIDASE IN GÖTTINGEN MINIATURE PIGS UNDERGOING REPEATED EXPOSURE TO EXCESS PRESSURE.

HYPOTHESIS.

This estimation was performed in conjunction with the estimation of urinary hydroxyproline excretion outlined in Experiment 4. The ability of the assay to detect localised bone necrosis had already been studied in Experiment 2. The aim of measurements in miniature swine undergoing repeated hyperbaric exposures was to see if the serum levels were affected by hyperbaric exposure (which would render the assay inapplicable for possible early detection of dysbaric osteonecrosis) and also to monitor the serum levels in any minipig developing dysbaric osteonecrosis.

Venepuncture in miniature swine is technically difficult. Special restraining devices have been invented (Tegeris et al., 1966) and in some studies blood from ear veins has been used. Powell et al. (1974) commented on the difficulty of repeated sampling from the ear veins. It was therefore decided that indwelling intravenous cannulae should be used for the sampling thus obtaining repeated samples over a long period of time without restraining or anaesthetising the miniature swine. There was only one account of this having been done before (Stegall and Smith, 1976) and these authors had maintained patency of an indwelling right atrial cannula for up to four weeks.

MATERIALS AND METHODS

The Broviac Parenteral Alimentation Catheter was purchased direct

from the manufacturer, Evergreen Medical Products, Inc. (Medina, Wa., U.S.A.). Each catheter is a 90cm. long barium impregnated silicone rubber tubing 0.10mm. I.D. x 0.22mm. O.D. with a silicone rubber sheath 36cm. long and 0.33mm O.D. leading from the external Luer Lock connector to a Dacron felt cuff. This cuff is designed to allow tissue ingrowth for catheter fixation and to act as a bacteria barrier.

For operation, the minipig was bathed and then anaesthetised. Anaesthesia was induced and maintained with a mixture of oxygen/nitrous oxide/halothane administered via a snout-shaped plastic mask specially made for this purpose. Smaller animals were suspended by their hind limbs as described by Strunin et al. (1977) but larger animals were held and anaesthetised in the box used for transporting them to and from the compression chamber.

The anaesthetised minipig was laid on the operating table on its left side and the right side and dorsum of the neck shaved and prepared with a solution of Savlon (1 part: 20 parts methyl alcohol). The head and body were excluded by drapes to obtain a sterile field and the operator wore gown and gloves.

A skin incision was made transversely about two to three cm. caudal to the angle of the mandible and the platysma was divided in the line of the skin incision. Very little bleeding was encountered. The strap muscles of the neck were split longitudinally by blunt dissection and retracted to reveal a jugular vein lying in areolor tissue immediately deep to the muscles. After passing ligatures beneath the vein a suitably sized tributary was incised with a sharp pointed blade (Schwann-Morton No. 11) and cannulated with the silastic catheter (cut to a length judged to reach the right side of the heart) up to the dacron felt cuff. Ligatures were then

tied, taking care not to occlude the lumen of the soft and easily compressed silastic catheter.

A subcutaneous tunnel was made from this skin incision to the dorsum of the neck and a large pair of artery forceps used to pass the external end of the catheter bearing the luer lock connector through the tunnel to exit via a 1cm. longitudinal incision on the midline of the dossum of the neck. The patency of the catheter was tested and 300 mg. penicillin (Crystapen brand for parenteral use) in 2 ml. water for injection inserted followed by a 2 ml. heparin saline lock to maintain patency. (Normal saline 100 mls. + 5000 units Heparin). The wounds were closed with interrupted 1 linen sutures to the platysma and interrupted 0 silk sutures to the skin. The catheter was secured to the dorsum of the neck with several 0 silk sutures.

The minipig was returned to its pen before awaking and sutures removed from the neck wound the next time the animal had an anaesthetic (e.g. for radiographic or scintigraphic examination).

Daily blood samples were then taken from the minipigs for as long as possible. All samples were taken between $08.^{15}$ - $09.^{15}$ h. The minipigs were fed while the samples were taken and experienced no discomfort. 1 ml. of blood was removed from the catheter and discarded, then a 4 ml. sample withdrawn each day. 300 mg. penicillin in 2 ml. water for injection was inserted followed by 2 ml. of heparin saline. The samples were centrifuged immediately after withdrawal, the serum collected by pipetting, and the samples stored at - $20^{\circ}C$. until assayed.

The assay was performed by Clin Path Services Ltd. as it was for Experiment 2.

The details of hyperbaric exposures of these minipigs are given

in Experiment 3.

RESULTS.

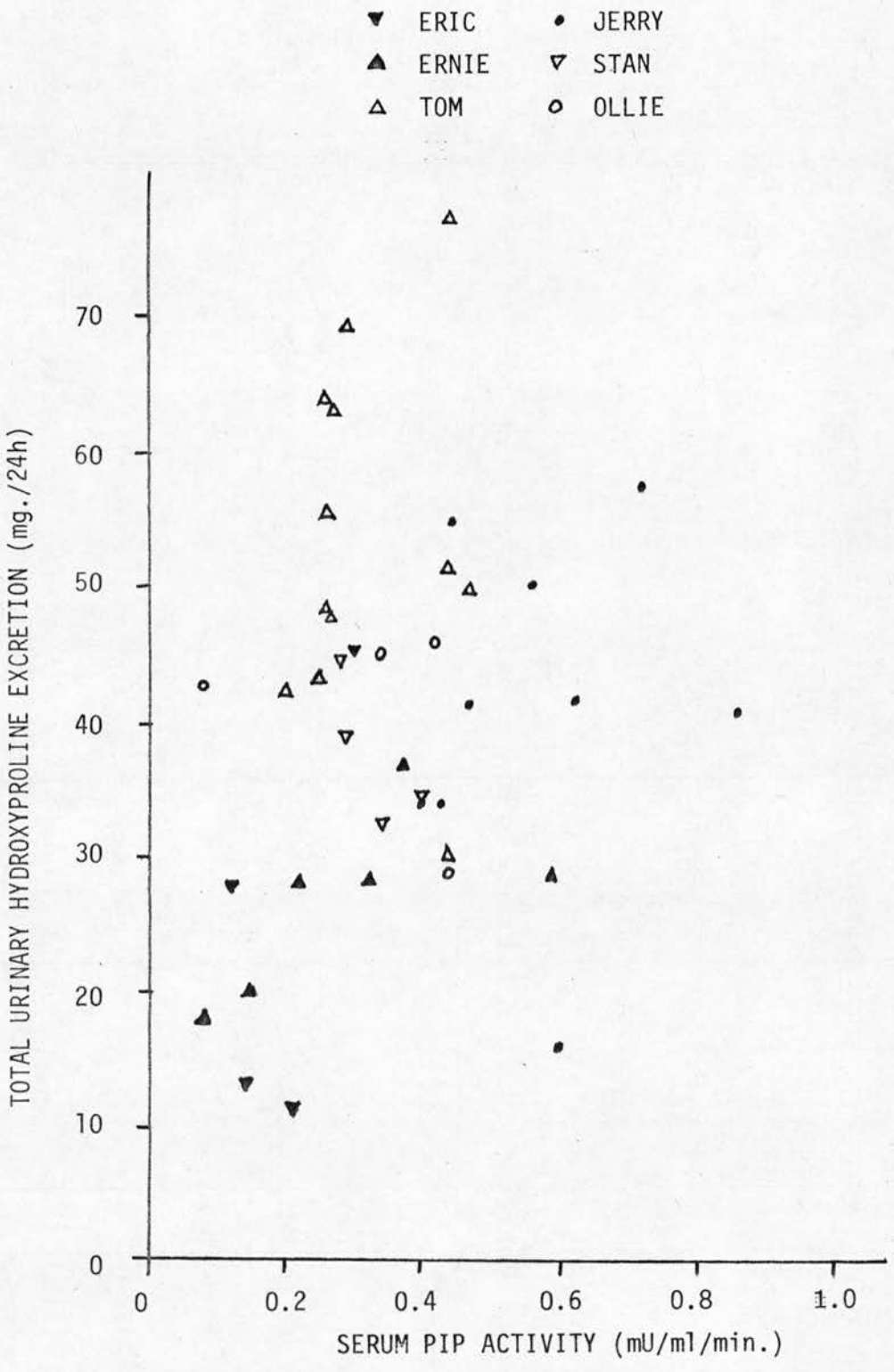
There were surprisingly few problems with the indwelling silastic catheters. Minipig Eric developed a wound infection (see Experiment 3). Withdrawal of samples on this minipig (which was the first minipig to undergo operation) was erratic and when the catheter was removed it was found that the intravenous portion had been left too long and had become kinked. Care was therefore taken to avoid this technical error in the remaining animals.

The detailed serum proline imino peptidase levels are included in Table 7 of Experiment 4, to permit comparison with the 24 hour total urinary hydroxyproline excretion estimations being collected at the same time. Further comparison is provided by Fig. 20, which compares the 3 day averaged 24 hour total urinary hydroxyproline excretion estimations with the serum PIP level on the middle day of the days used for the urinary estimations. Visual inspection suggests there is not a correlation.

DISCUSSION

There were no particular technical problems with this experiment. Before starting, concern was expressed in case the minipigs would be irritated by the catheter emerging on the dorsum of the neck and try and scratch through the soft silastic against the cage walls with the possibility of blood loss if this happened during the night. This did not happen. The catheters remained patent for up to twelve weeks in the six unrestrained miniature swine. This was an even longer period than expected as Stegall and Smith (1976) report removing a

FIG. 20. COMPARISON BETWEEN SERUM PROLINE IMINO PEPTIDASE ACTIVITY AND 24 HOUR TOTAL URINARY HYDROXYPROLINE EXCRETION IN MINIATURE SWINE



catheter 4 weeks post-surgery and finding that tissue had penetrated the Dacron felt cuff. This would imply that this was the longest period for which catheters remained in situ in their experiment.

It was expected that no positive results in respect of elevation of serum proline imino peptidase levels would be recorded in this experiment. This was because of the apparent inability of the assay to detect areas of artifically induced bone and marrow necrosis (see Experiment 2) plus the failure of any of the six minipigs studied to develop dysbarism-related osteonecrosis (see Experiment 3). The results show relatively little variation, with the exception of one freak result of 1.75 mU/ml/min on 19th April on minipig Eric. Review of the compression chamber log does not reveal any decompression sickness in this minipig at this time. The laboratory has checked the result and it remains unexplained. All other results fell within the range of 0.06 - 1.02 mU PIP/ml/min, and the values recorded prior to any hyperbaric exposure fell within the range 0.08 - 0.85 mU PIP/ml/min (pre-exposure values were not available on two minipigs).

There does not appear to be a correlation between the serum PIP levels and the total urinary hydroxyproline excretion. Whiteley et al. (1976) did find that the serum PIP correlated with other indices of collagen breakdown (alkaline phosphatase and 24 hour total urinary hydroxyproline excretion) and the failure to find any correlation in the present experiment seems most likely to be a reflection of inaccuracy in the urine collections, despite the precautions used as detailed in Experiment 4.

CONCLUSIONS

Indwelling silastic right atrial catheters can be maintained

in unrestrained miniature swine for up to 12 weeks with daily checks of their patency.

No change was detected (compared with pre-exposure levels) in the serum proline imino peptidase activity of six miniature swine following multiple hyperbaric exposures.

No change in the serum proline imino peptidase activity was found following episodes of acute decompression sickness in miniature swine.

CHAPTER 7. EXPERIMENTS CONCERNING THE EARLIER DIAGNOSIS OF AN ESTABLISHED LESION AND REPAIR PROCESSES.

EXPERIMENT 6 : SKELETAL SCINTIGRAPHY AND RADIOLOGY IN GÖTTINGEN MINIATURE PIGS UNDERGOING REPEATED EXPOSURE TO EXCESS PRESSURE.

HYPOTHESIS.

Radiographic examination is at present the earliest method of detecting caisson disease of bone in man. Radiographic abnormalities have also been reported in relation to bone necrosis produced after hyperbaric exposure in miniature swine (Smith and Stegall, 1974; Gregg, 1977). The earliest time from first hyperbaric exposure that radiographic changes have been evident appears to be six weeks (Stegall et al., 1973). Smith and Stegall (1974) also found areas of "advanced bone necrosis" at autopsy which had not been seen on earlier radiographs.

Scintigraphy seems to have been only rarely performed in miniature swine. The main report seems to be that of Eckelman et al. (1974). The only report of a 'hot spot' of increased uptake in association with an area of bone necrosis in a miniature pig appears to be that of Gregg (1977). Gregg states that he commenced scintigraphy in miniature swine only after it had shown itself to be a technique which detected experimental osteonecrosis in rabbits earlier than radiography. All the miniature swine he studied had already had numerous hyperbaric exposures at this stage in his research and although the one minipig with bone necrosis did demonstrate positive scintigraphy it also had radiographic abnormality and no conclusion could be drawn about whether the scintigraphic appearance had become abnormal before the radiographic appearance.

However, in view of this positive finding it appeared well worthwhile to undertake a further study in miniature swine subjected to repeated hyperbaric exposure.

MATERIALS AND METHODS

The 6 minipigs used and the details of their ages, weights and hyperbaric exposures are discussed in detail in Experiment 3.

Skeletal scintigraphy and appropriate radiography was performed before each minipig commenced its series of hyperbaric exposures and at approximately six week intervals thereafter. A final set of scintigraphs and radiographs were obtained six weeks or longer after the minipigs' last hyperbaric exposure (except the one minipig which died during the experiment). For all the procedures in this experiment it was necessary to have the minipigs still and all the procedures were performed under general anaesthesia. After the first two occasions the radiographs and scintigraphs were performed on the same day, so that the general anaesthetic necessary for the radiographs could also be used for the intravenous injection of the skeletal imaging agent and the total number of anaesthetics for each minipig could therefore be reduced. The minipigs were anaesthetised with a mixture of halothane, nitrous oxide, and oxygen administered via a stout snout-shaped polythene mask as already described in Experiment 5.

All the radiographs were performed on a Watson MX4 unit using Ilford Rapid R xray film and Ilford Fast Tungstate Intensifying Screens. The minipig was first positioned on its back with the lower limbs held still by an assistant while a radiograph of the pelvis including both hips and proximal femora was exposed. This

was the most difficult view on which to obtain good definition of bone detail as fairly deep anaesthesia was required to avoid movement and as the animals increased in weight the soft tissues decreased the penetration of the xrays. The minipig was turned on to one side and the knee and shoulder nearest the xray table exposed with traction on the limb to remove the abdominal soft tissue shadow as much as possible. The minipig was then turned on to its opposite side and the procedure repeated. The minipig was kept anaesthetised until the radiographs had been developed and viewed by myself in case any were technically unsatisfactory.

All the scintigraphs were performed a minimum of three hours after an intravenous injection of 10 mCi of ^{99m} Tc labelled 1-ethylidene 1,1-hydroxidiphosphonate (EHDP) using commercial Osteoscan administered via an ear vein. If an indwelling intravenous cannula was present as part of Experiment 5 the EHDP was administered via the cannula. The equipment used for scintigraphy was the same as that used for the human studies in Experiment 9, an Ohio Nuclear Series 100 gamma camera with a converging collimator. The minipig was anaesthetised and laid on its side on a trolley and each limb in turn held next to the collimator while an appropriate count (usually 120k) was amassed. The count was pre-set for the right limbs and the same time pre-set for the left limbs (as described in Experiment 9). The scintigraphs were developed on Polaroid film (Kodak Ltd.)

In addition to the radiographs performed on the live minipigs, after autopsy the femora, tibiae, and humeri of each animal were dissected free of soft tissue and radiographed on a Watson MX4 unit using Ilfex film prior to their being frozen for sectioning.

RESULTS

Details of the performance of the scintigraphs and radiographs in relation to the series of hyperbaric exposures are given in Table 9. A specimen set of radiographs are reproduced in Figs. 21 to 25. The bone detail is not as well delineated on the smaller size of the photographic reproductions. This set of radiographs was performed when the minipig was 19 months old and shows that the epiphyses have not fused at that time. The scintigraphs performed on the same minipig on the same day are reproduced in Figs. 26 to 29. The radiographs of the excised bones of this minipig are reproduced in Figs. 30 and 31. Excellent detail of the trabecular structure was present in the radiographs of the excised bones of all six minipigs.

The reproducibility of the radiographic and scintigraphic techniques was satisfactory. One minipig had a prominent linea aspera in the distal femur on each side and this consitently showed an increased uptake of skeletal imaging agent on scintigraphy.

None of the radiographs or scintigraphs showed any abnormality, and there was no evidence of bone or marrow necrosis on the radiographs of the excised bones or on macroscopic examination of these bones.

DISCUSSION.

None of this group of minipigs developed bone or marrow necrosis. This is unfortunate as it would seem possible to find out if scintigraphy detects bone necrosis earlier than radiography more easily in animals than in humans. I anticipate that it will take several years before any results will be available from the survey set up in Experiment 9 in humans. I, therefore, feel that this experiment should be repeated using the techniques described.

The fact that the epiphyses of this group of minipigs had not fused,

TABLE 9. SCINTIGRAPHY AND RADIOGRAPHY OF MINIATURE SWINE IN RELATION TO HYPERBARIC EXPOSURES.

ERIC.

EVENT	DAYS SINCE FIRST HYPERBARIC EXP.	NUMBER OF DECOMPRESSIONS	HOURS AT 27 p.s.i.g.
PRE-EXPOSURE SCAN/XRAY	-17/-11	-	-
FIRST HYPER-BARIC EXPOSURE	0	-	-
SCAN/XRAY	39/44	21/23	119/128
SCAN/XRAY	81	39	215
SCAN/XRAY	151	49	267
SCAN/XRAY	207	56	300
LAST HYPER-BARIC EXPOSURE	191	56	300
FINAL SCAN/ XRAY	242	"	"

ERNIE.

PRE-EXPOSURE SCAN/XRAY	-17/-11	-	-
FIRST HYPER-BARIC EXPOSURE	0	-	-
SCAN/XRAY	39/44	22/24	125/134
SCAN/XRAY	81	35	194
LAST HYPER-BARIC EXPOSURE	60	"	"
DEATH	87	"	"

TABLE 9 continued

SCINTIGRAPHY AND RADIOGRAPHY OF MINIATURE SWINE

IN RELATION TO HYPERBARIC EXPOSURES.

STAN.

EVENT	DAYS SINCE FIRST HYPERBARIC EXP.	NUMBER OF DECOMPRESSIONS	HOURS AT 27 p.s.i.g.
PRE-EXPOSURE SCAN/XRAY	-4	-	-
FIRST HYPER-BARIC EXPOSURE	0	-	-
SCAN/XRAY	45	25	140
SCAN/XRAY	unwell		
SCAN/XRAY	136	35	196
SCAN/XRAY	192	51	255
LAST HYPER-BARIC EXPOSURE	218	59	282
FINAL SCAN/XRAY	249	59	282

OLLIE.

PRE-EXPOSURE SCAN/XRAY	-4	-	-
FIRST HYPER-BARIC EXPOSURE	0	-	-
SCAN/XRAY	45	25	140
SCAN/XRAY	87	40	223
SCAN/XRAY	136	46	253
SCAN/XRAY	192	76	364
LAST HYPER-BARIC EXPOSURE	218	84	393
FINAL SCAN/XRAY	249	84	393

TABLE 9 continued

SCINTIGRAPHY AND RADIOGRAPHY OF MINIATURE SWINE IN RELATION TO HYPERBARIC EXPOSURES.

TOM

EVENT	DAYS SINCE FIRST HYPERBARIC EXP.	NUMBER OF DECOMPRESSIONS	HOURS AT 27 p.s.i.g.
PRE-EXPOSURE SCAN/XRAY	- 12	-	-
FIRST HYPER-BARIC EXPOSURE	0	-	-
SCAN/XRAY	51	27	156
SCAN/XRAY	95	49	263
LAST HYPER-BARIC EXPOSURE	128	63	317
FINAL SCAN/ XRAY	165	"	"

JERRY

EVENT	DAYS SINCE FIRST HYPERBARIC EXP.	NUMBER OF DECOMPRESSIONS	HOURS AT 27 p.s.i.g.
PRE-EXPOSURE SCAN/XRAY	-12	-	-
FIRST HYPER-BARIC EXPOSURE	0	-	-
SCAN/XRAY	51	27	156
SCAN/XRAY	95	48	260
LAST HYPER-BARIC EXPOSURE	128	64	320
FINAL SCAN/ XRAY	165	"	"

Fig. 21. Minipig Eric. Radiograph Right humerus. The 'scalloped' endosteal appearance on the distal shaft (arrows) is normal.

Fig. 22. Minipig Eric. Radiograph Left humerus.

Fig. 23. Minipig Eric. Radiograph pelvis and both proximal femora.

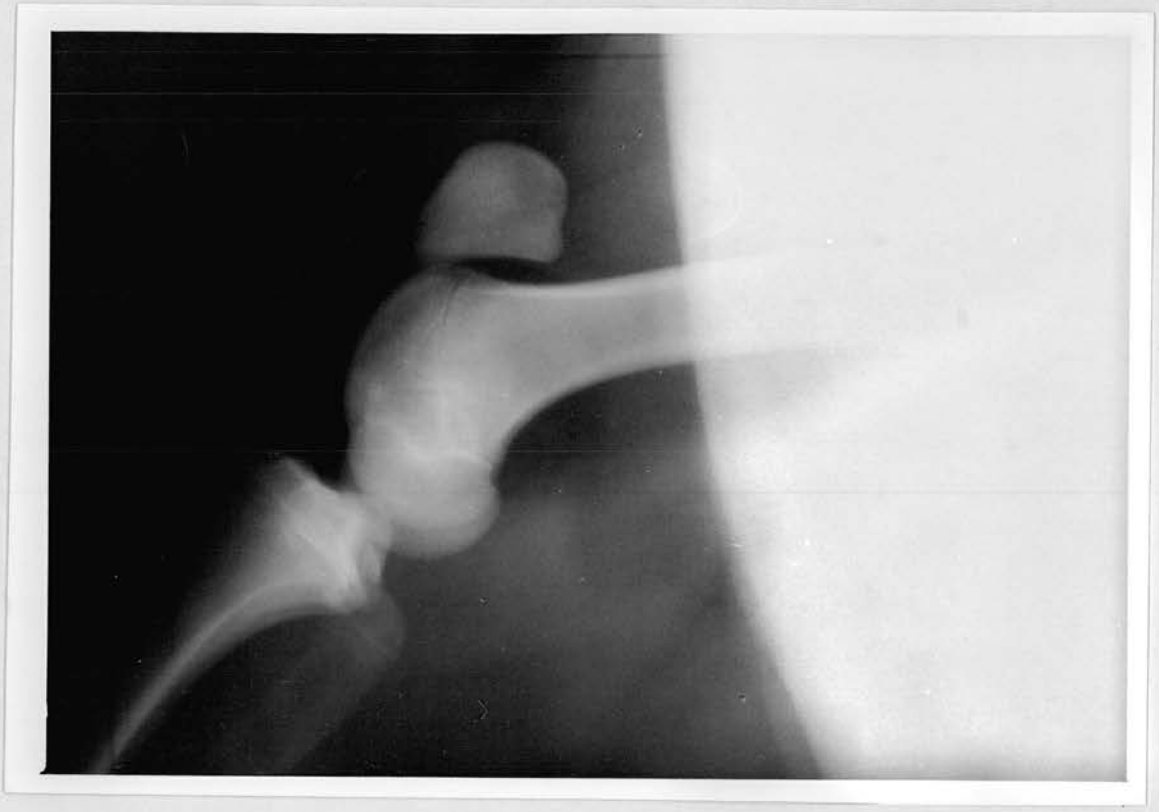


Fig. 24 Minipig Eric. Lateral radiograph of Right knee

Fig. 25. Minipig Eric. Lateral radiograph of Left knee.

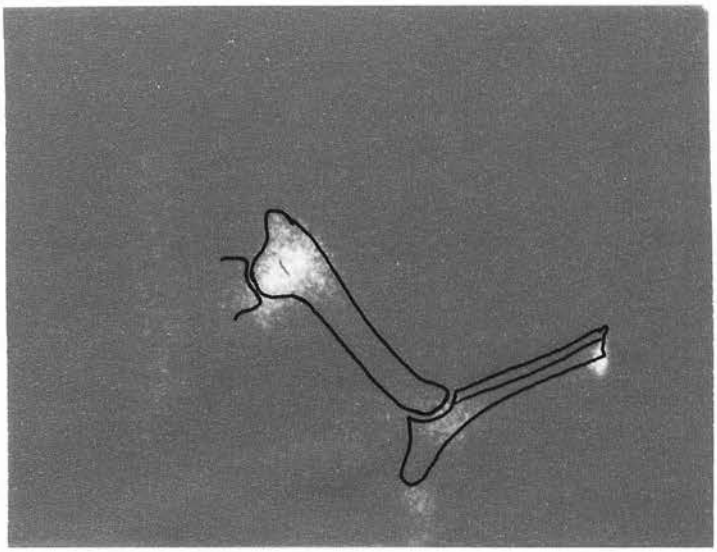


Fig. 26 Minipig Eric. Scintigraph of Right humerus.

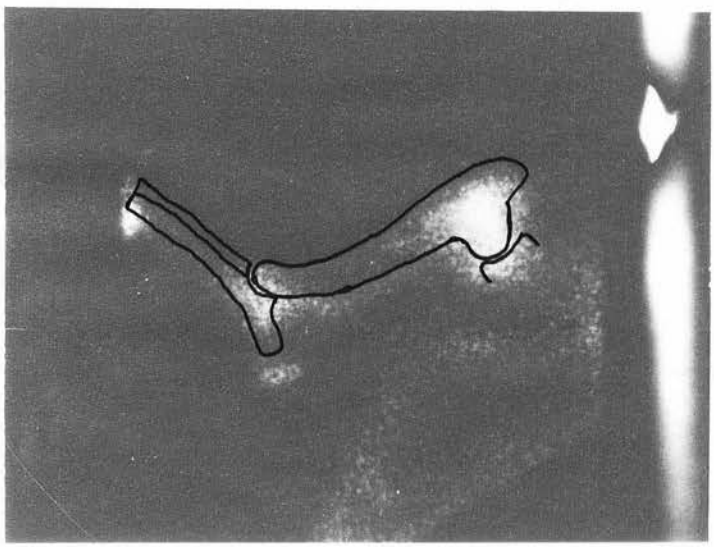


Fig. 27. Minipig Eric. Scintigraphy of Left humerus.

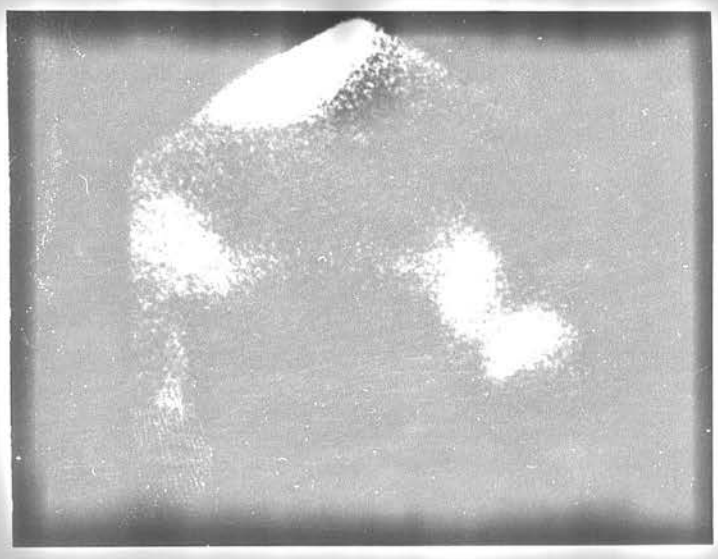


Fig. 28. Minipig Eric. Scintigraph of Right femur.

Fig. 29. Minipig Eric. Scintigraph of Left femur.

Fig. 30. Minipig Eric. Radiograph of excised right humerus, femur and tibia.

Fig. 31. Minipig Eric. Radiograph of excised left humerus, femur and tibia.

probably because they had been castrated, made me wonder if this was a possible reason why osteonecrosis had failed to develop using the same exposures and decompression as reported by Gregg (1977). He kindly allowed me to review the radiographs of his miniature swine, which had also been castrated, and I found that the epiphyseal lines were clearly visible at two years of age on his animals. It is therefore not essential for the epiphyses to have fused for osteonecrosis to develop in miniature swine following hyperbaric exposure, as one of Gregg's minipigs developed osteonecrosis.

The minipigs did not have any visible ill-effects from the numerous anaesthetics necessary for the performance of this experiment.

Reports in the literature of almost universal radiographic changes in miniature swine two to three months following a series of similar hyperbaric exposures (Stegall et al., 1974) have not been confirmed. No radiographs are available in this publication to indicate what radiographic findings were interpreted as showing osteonecrosis in 14 out of 15 minipigs studied. Other publications including radiographs from the same research group (Smith and Stegall, 1974 ; Stegall and Smith, 1976) show sclerotic rings in the proximal femur. This appearance was not seen in the present study.

CONCLUSIONS.

The techniques of this experiment appear satisfactory. No positive radiographs or scintigraphs were obtained in the limb bones of the 6 miniature swine studied.

EXPERIMENT 7. SKELETAL SCINTIGRAPHY OF RABBITS WITH ARTIFICIALLY INDUCED OSTEONECROSIS.

HYPOTHESIS.

This is not an original experiment. The data presented became available as part of Experiment 8. In view of the increasing use in other centres of skeletal scintigraphy in human subjects with suspected osteonecrosis it seemed important to report these results confirming the original work of Gregg (1977) which indicated that skeletal scintigraphy might be a possible method for the early detection of osteonecrosis.

Gregg used the procedure for producing osteonecrosis outlined in Experiment 2. He injected glass microspheres into the iliac artery of New Zealand White rabbits to produce bone and marrow necrosis of the femur. Scintigraphy and radiography were performed three weeks and thirteen weeks after operation. Scintigraphy detected most of the lesions shown on subsequent histological examination as early as three weeks after operation. In contrast, very few radiographic changes were visible even thirteen weeks after operation.

For the present work skeletal scintigraphy was performed on rabbits six weeks after the same operation and the positive findings compared with subsequent histology. From reviewing the histological findings at different times after this operation (Cox, 1974 and unpublished personal observations) six weeks appeared to be a time when the repair processes were well established. I, therefore, hoped that skeletal scintigraphy at this time would detect all the lesions of bone necrosis found histologically.

MATERIALS AND METHOD.

Fourteen adult female New Zealand White rabbits weighing 3.1 to

5.2 Kg. were used. Each rabbit underwent the operation described in Experiment 2 at which glass microspheres 50 - 70 um diameter were injected into the right iliac artery. No antibiotic prophylaxis was used. There were no cases of wound infection.

Six weeks after operation skeletal scintigraphy was performed on each rabbit. 5mCi of ^{99m}Tc - labelled EHDP (1-hydroxiethylidene 1,1- diphosphonate) was injected intravenously into a marginal ear vein. Three hours later the rabbit was lightly anaesthetised with veterinary pentobarbitone sodium ('Sagatal'; May and Baker) and placed supine with the hind limbs secured by tapes. Scintigraphs were recorded from each femur using an Ohio Nuclear gamma camera with a pinhole collimator of 4 mm aperture. Care was taken to measure the distance of the rabbit femur from the collimator to make this the same for each limb. Each femur was counted for the same time interval (set electronically by the gamma camera equipment to 0.01 sec.) The scintigraphs were recorded on Polaroid Land film type 87. (polaroid Corporation; Cambridge, Mass.) Before the animal was killed or histological sections were examined a written interpretation of these scintigraphs was made and areas of increased uptake of radioisotope in the right femur not present in the left (control) femur were recorded.

The rabbits were killed zero to nine weeks later as part of experiment 8. After a thin slab of bone had been removed from the proximal femur the entire bone was submitted for routine histological examination as already described in experiment 1.

RESULTS.

The results of scintigraphic and histological examinations are shown in Table 10. In sixteen sites showing positive scintigraphy bone necrosis and new bone formation were confirmed and one rabbit

TABLE 10. SCINTIGRAPHY AND HISTOLOGY OF RABBIT RIGHT FEMORA AFTER ARTERIOLAR BLOCKADE.

R.	AREAS OF INCREASED UPTAKE ON SCINTIGRAPHY.	ABNORMALITIES ON HISTOLOGY (LONGITUDINAL CORONAL SECTIONS).
3:1.	Upper Shaft	Upper Shaft: Large area marrow necrosis. Some dead trabecular bone with appositional new bone formation. Osteocyte loss inner $\frac{2}{3}$ of cortex. Lower Shaft: Extensive lesion within marrow. Necrosis with marrow fibrosisand new bone formation around it. Periosteal reaction.
3:2.	None	None
3:3.	Head Greater trochanter. Upper shaft	Head and neck necrotic (bone and marrow) with appositional new bone formation. Greater trochanter necrotic (bone and marrow) with appositional new bone formation. Proximal shaft: Marrow fibrosis. One cortex has osteocyte loss.
3:4.	Head Greater trochanter and proximal shaft. Mid-shaft.	Head. Entire epiphysis dead and remodelling. Patchy necrosis trabecular bone and marrow at base of greater trochanter with some appositional new bone. Mid-shaft. Massive periosteal new bone (almost an exostosis) but no obvious cortical or marrow necrosis.

TABLE 10. SCINTIGRAPHY AND HISTOLOGY OF RABBIT RIGHT FEMORA AFTER ARTERIOLAR BLOCKADE (continued)

R.	AREAS OF INCREASED UPTAKE ON SCINTIGRAPHY.	ABNORMALITIES ON HISTOLOGY (LONGITUDINAL CORONAL SECTIONS).
3:5.	Upper shaft - possibly extending into greater trochanter Doubtful positive just prox. to condyles.	Extensive cortical and marrow necrosis proximal shaft. Some periosted and endosteal new bone. Osteocyte loss inner cortex distal shaft. No other changes seen in distal shaft.
3:6.	Epiphyses not fused? Head Greater trochanter/prox. shaft	Epiphysis not fused. Osteocyte loss of cortex shaft (full length), Focal marrow fibrosis proximal shaft with some new bone formation. Head normal.
3:7.	Upper shaft. Mid shaft. Doubtful positive lower shaft.	Osteocyte loss femoral neck cortex. O steocyte loss inner $\frac{2}{3}$ cortex mid shaft with endosteal new bone. Some marrow fibrosis also at this site.
3:8.	None	Epiphysis of head dead with appositional new bone formation.
3:9.	Lower shaft (condyles) Upper half of shaft	Normal.
3:10.	None	Necrosis $\frac{2}{3}$ of head epiphysis. Small amount appositional new bone.
3:11.	Head Mid shaft Condyles	Head - one remodelling dead trabeculum. Shaft - patchy cortical necrosis and marked periosteal reaction.
3:12.	None	Head : Epiphysis completely dead and remodelling.

TABLE 10.SCINTIGRAPHY AND HISTOLOGY OF RABBIT RIGHT FEMORA AFTER ARTERIOLAR BLOCKADE (continued)

R.	AREAS OF INCREASED UPTAKE ON SCINTIGRAPHY.	ABNORMALITIES ON HISTOLOGY (LONGITUDINAL CORONAL SECTIONS).
3:13.	Head and upper shaft	Small area cortical necrosis near base greater trochanter. Limited endosteal new bone.
3:14.	Upper shaft. Mid shaft. Lower shaft (very marked)	Massive necrosis of head and entire shaft with large amount new bone formation. Degenerative cartilage hip and knee joints.

TABLE 11.A.

CORRELATION OF SCINTIGRAPHY WITH DEAD BONE SEEN HISTOLOGICALLY

		OSTEONECROSIS.	
		POSITIVE	NEGATIVE
SCINTIGRAPHY	POSITIVE	17	4
	NEGATIVE	4	1

TABLE 11.B.

CORRELATION OF SCINTIGRAPHY WITH NEW BONE FORMATION SEEN HISTOLOGICALLY.

		NEW BONE FORMATION	
		POSITIVE	NEGATIVE
SCINTIGRAPHY	POSITIVE	16	5
	NEGATIVE	4	1

with normal scintigraphy had no abnormality on histological examination. However, in four instances a positively interpreted scintigraph did not show any histological abnormality and in four sites bone necrosis and new bone formation was found when the scintigraph had shown no abnormality at that site.

One site of positive scintigraphy showed osteonecrosis but no new bone formation. I suspect this was a chance finding upon the particular plane of the histological section and that examination of serial sections would have shown areas of new bone formation. In all other instances dead bone and new bone formation were closely allied and had the same relationship to positive scintigraphic findings (Table 11). This will be discussed in Experiment 8.

DISCUSSION.

Even though some of the rabbits in this experiment were not sacrificed until several weeks after scintigraphy was performed the majority of the areas of osteonecrosis showed positive scintigraphy (17 out of 21) and scintigraphy only gave rise to four false positives. It is possible that these false positives were areas completely remodelled in the weeks between scintigraphy and sacrifice but I think this is very unlikely. One was a femoral head (rabbit 6) which appeared normal only three weeks later, and two positive scintigraphs were at the level of the femoral condyles, which is a site seemingly almost immune to osteonecrosis following this particular operative technique.

This level of false positives and false negatives is very similar to that recorded by Gregg (1977). The results appear to confirm his impression that skeletal scintigraphy may be a useful early indicator

of experimentally produced bone and marrow necrosis in rabbits.

CONCLUSION.

Skeletal scintigraphy performed six weeks after the experimental induction of osteonecrosis in the femora of adult New Zealand White rabbits by the technique described shows an increased uptake of radio-isotope in the majority of areas subsequently shown to be the site of osteonecrosis.

EXPERIMENT 8 : AUTORADIOGRAPHIC LOCALISATION OF BONE-SEEKING RADIOISOTOPES IN ARTIFICIALLY INDUCED OSTEONECROSIS IN RABBITS.

HYPOTHESIS

The ultimate fate of diphosphonate skeletal imaging agents appears to be either excretion in the urine or incorporation into the hydroxyapatite crystal lattice of bone, possible during formation of the lattice. Diphosphonates seem to interfere with the geometry of the lattice and prevent addition of further hydroxyapatite. They have, therefore, been used to treat myositis ossificans progressiva and on a trial basis Paget's disease of bone.

However, it is not universally agreed that an increased uptake of radioisotope on skeletal scintigraphy is related to new bone formation. Some workers believe it is primarily related to an increase in bone blood flow (e.g. Hughes et al., 1977). Others have drawn attention to the increased amount of osteoid tissue in conditions associated with positive scintigraphy and confirmed the avidity of bone matrix for skeletal imaging agents in in vitro experiments (Rosenthall and Kaye, 1976). There is some autoradiographic evidence that ^{99m}Tc - polyphosphate localises in mineralised bone and not adjacent osteoid (Tilden et al., 1973; Galasko, 1975) but these studies were performed on specimens removed a minimum of three hours after administration of the skeletal imaging agent.

To understand what the significance of a 'hot-spot' of increased radioisotope uptake is on skeletal scintigraphy it is necessary to know what this represents. This is perhaps especially so in considering localised areas of dead bone. An interpretation of this increased uptake as indicating an increase in local blood flow might lead to a

different prognosis and management from an interpretation of excess unmineralised osteoid. This experiment sets out to investigate if the uptake of ^{99m}Tc-labelled diphosphonate in areas of experimentally-produced osteonecrosis can be localised autoradiographically and if this can be done soon enough after administration of the radioisotope to see any intermediary steps in its uptake into the hydroxyapatite lattice.

The preliminary requirement for an experiment of this nature is to select an appropriate β particle emitting radioisotope. The resolution of autoradiographs depends on the energy of the β particles and the thickness of the section of the specimen Tritium is the most frequently used isotope in biological studies because it is of low energy and gives good resolution. Its particles have an energy of 0.018 MeV and in biological materials 80% of these have a range of less than 1 μm and only 1% achieve a range of 3 μm (Caro, 1962). It would be possible to label a diphosphonate imaging agent with Tritium but Rosenthall and Kaye (1976) performed an experiment which suggested that a label attached to the diphosphonate complex may not localise in the same material as the ^{99m}Tc label which gives rise to the positive scintigraphy.

Fortunately, ^{99m}Tc has itself a number of emissions of an energy theoretically suitable for converting silver halide in nuclear emulsions, in addition to its gamma emission so suitable for scintigraphy, (Table 12). In the figure the emissions thought to be useful are marked with an asterisk. Successful autoradiography using ^{99m}Tc has been reported by several workers (listed in Table 13). Many of these reports indicate that they found it necessary to prepare the

TABLE 13. ^{99m}Tc Output Data (Willis et al., 1977)

Radiation	Mean number per 100 disintegrations	Mean energy (KeV)
γ-1	0.00	2.1
M internal conversion electron, γ-1	98.60	1.7
γ-2	88.30	140.5
* K internal conversion electron, γ-2	8.83	119.5
* L internal conversion electron, γ-2	1.09	137.9
* M internal conversion electron, γ-2	0.36	140.1
γ-3	0.03	142.7
* K internal conversion electron, γ-3	0.96	121.7
* L internal conversion electron, γ-3	0.30	139.9
* M internal conversion electron, γ-3	0.10	142.3
K-1 X-ray	4.31	18.4
K-2 X-ray	2.16	18.3
K-1 X-ray	1.03	20.6
K-2 X-ray	0.18	21.0
* L X-ray	0.81	2.4
* KLL Auger electron	1.49	15.5
* KLX Auger electron	0.55	17.8
* KXY Auger electron	0.07	20.2
LMM Auger electron	10.60	1.9
MXY Auger electron	123.00	0.4

TABLE 13. Autoradiographic Localisation of ^{99m}Tc-labelled compounds

Compound	Tissue	Author
^{99m}Tc-Sodium pertechnate	Human brain tumour (acoustic neuroma)	Baum & Rothballer
^{99m}Tc-sulphur colloid	Liver (mice)	Chaudhuri, et al.(1973)
^{99m}Tc-pertechnate	Gastric mucosa (cats)	Meier-Ruge and Fridrich (1973)
^{99m}Tc-polyphosphate	Human femoral head	Tilden, et al. (1973)
^{99m}Tc-Stannous glucoheptonate	Kidney (rat)	Willis, et al. (1977)
^{99m}Tc-Stannous Dimercaptosuccinate	Kidney (rat)	Willis, et al. (1977)
^{99m}Tc-polyphosphate	Tibia (rabbit)	Galasko (1975)
^{99m}Tc-tetracycline	Cells from tissue culture (mammal)	Dewanjee (1975)

material for autoradiography rapidly because of the short half-life of ^{99m}Tc. (6.1 hours). This short half-life means that only a short exposure is merited compared with the usual three month exposure when preparing autoradiographs using Tritium labels (half-life 12-26 years.) The resolution of ^{99m}Tc autoradiography for a thin specimen and emulsion layer was calculated by Willis, et al. (1977) to be 2.3 μm resolution compared with the 0.35 μm resolution of Tritium reported in similar circumstances (Salpeter et al., 1974).

There are no reports of autoradiographic localisation of skeletal imaging agents in instances of bone necrosis with increased uptake of these agents on scintigraphy. It was, therefore, decided to try and adapt the technique of Tilden et al. (1973) using ^{99m}Tc - labelled polyphosphate for microautoradiography to investigate areas of bone necrosis showing positive scintigraphy.

I appreciated that this would require modification of the published techniques. It would be essential to have satisfactory reproduction on the microscope slides of the bone cells, bone matrix, and bone mineral, as the radioactivity might be in any of these at different times. This is not easy, as to cut thin sections on an ultramicrotone the specimen has to be embedded in a material of similar hardness to itself (otherwise the cutting blade "chatters" when it crosses the specimen). Bone mineral is very hard but bone cells are soft and easily deformed and disrupted by infiltration with a hard embedding medium.

I was intending to use adult female New Zealand white rabbit with bone necrosis produced by the intra-arterial injection of glass microspheres as described by Cox (1973, 1974) and review of the literature of ultramicrotome sectioning of undecalcified bone (Table

Table 14. Sectioning of undecalcified bone on ultramicrotomes

Material	Author	Embedding medium	Microtome	Knife blade	Section thickness
Young rats	Anderson and Parker (1966)	Methacrylate Araldite	Porter-Blum	-	"thin"
Newborn mice	Bernard (1969)	Epon (Luft's method)	-	diamond	"thin"
Fowl embryo	Boothroyd (1964)	Araldite	Huxley Cambridge	diamond	400 Å
Dogs (micro-dissection of single haversian systems)	Cooper et al. (1966)	Methacrylate Epon 812	Porter-Blum	diamond	700 - 1200 Å
Human (fibrous dysplasia lesions)	Dudley and Spiro (1961)	Araldite	Porter-Blum	glass diamond	2 µm "thin"
Young rats (minced growth plate)	Matthews et al. (1968)	Epon	Porter-Blum	diamond	0.5 µm
Human (adult)	Tilden et al. (1973)	Luft's method	Porter-Blum	glass	1.0 µm

14) showed that there had been very little work using mature adult bone. The photomicrographs in these published reports usually showed poor cellular details unless the bone had been soft enough to allow more conventional fixation and embedding - for example, Anderson and Parker (1966) write that the femora they studied were split longitudinally with a razor blade. This would certainly not be possible in an adult rabbit. In addition to this, many of the described methods require a time-course of preparation too long to be suitable for the proposed study. Tilden, et al. (1973) stated that they used Luft's method (Luft, 1961) but modified the embedding part of the procedure so that the preparation time to exposure of the thin sections to autoradiographic emulsion was reduced to 14 hours. In his 1961 paper Luft described embedding procedures using both Araldite and Epon 812 and it was not clear which of these Tilden and co-workers had used. However, they showed that this short a preparation time was possible.

Local experience of embedding materials for electron microscopy showed that many workers had changed in recent years to using Spurr resin for routine work with biological materials. This epoxy resin was described by Spurr in 1969 and has a much lower viscosity than other epoxy resins This seemed a desirable property for infiltrating bone blocks since the resin might penetrate marrow Haversian systems. Rather surprisingly, no references could be found to the use of this resin for embedding undecalcified bone. By trial and error we developed a technique for making it of suitable hardness to allow sectioning of articular cartilage and trabecular bone, but we could not make it hard enough to embed lamellar bone. This does not matter, however, as the new bone formation seen histologically around areas of marrow necrosis and as appositional new bone on

dead trabeculae could still be studied, even if the endosteal and periosteal reaction to cortical necrosis could not.

The sections of trabecular bone we obtained with the technique to be described showed excellent cellular, osteoid, and mineral detail, and the preparation time using Spurr resin was therefore examined carefully and reduced without any deleterious effect so that it could become a suitable preparation for ^{99m}Tc microautoradiography of undecalcified bone.

MATERIALS AND METHOD.

The 14 adult female New Zealand White rabbits studied in experiment 7 provided the basic material for this experiment. Those rabbits with an increased uptake of radioisotope in the proximal femur (neck and trochanteric region) six weeks after operation were used. Examples of the typical positive scintigraphic appearances in this region are shown in Figs.32 and 33. This area proved to be suitable for study because the marrow cavity of the rabbit femur is fluid and microscope sections cannot be prepared from it without prior fixation or freezing. In contrast, the cancellous bone of the proximal femur could be cut without prior preparation and without the intertrabecular marrow flowing out of position. This was important as preparation time had to be reduced to a minimum because of the short half-life of ^{99m}Tc

1 - 3 hours before each rabbit for this experiment was sacrificed it received an intravenous injection of 5m Ci of ^{99m} Tc labelled methylene diphosphonate (MDP) into a marginal ear vein. Each rabbit was killed by an overdose of intravenous barbiturate anaesthetic. The time of death was determined by the availability of the personnel and equipment required for parts of the experiment and varied from

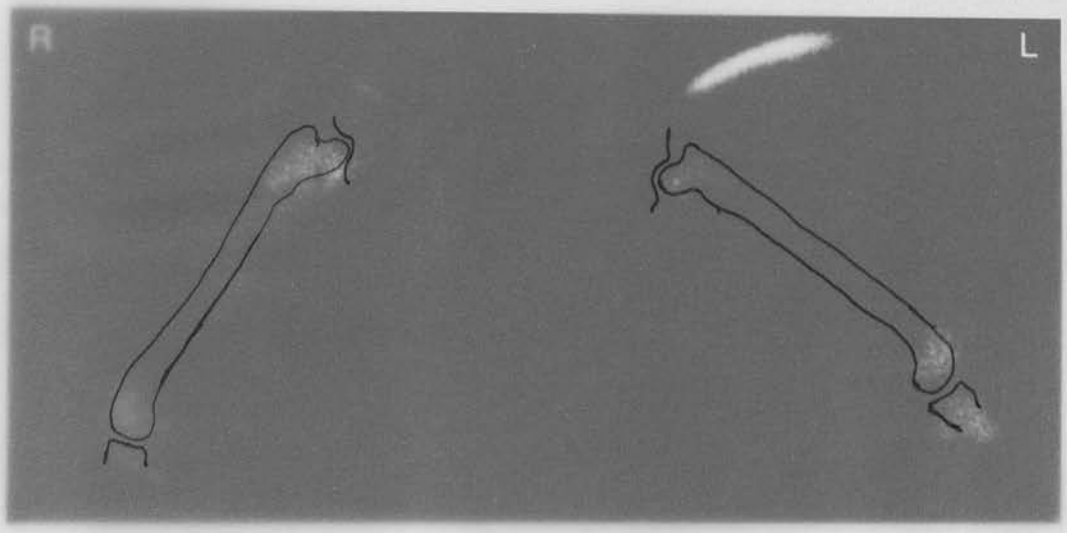


Fig. 32. Typical scintigraph showing increased uptake of radioisotope in right proximal femur (see text).

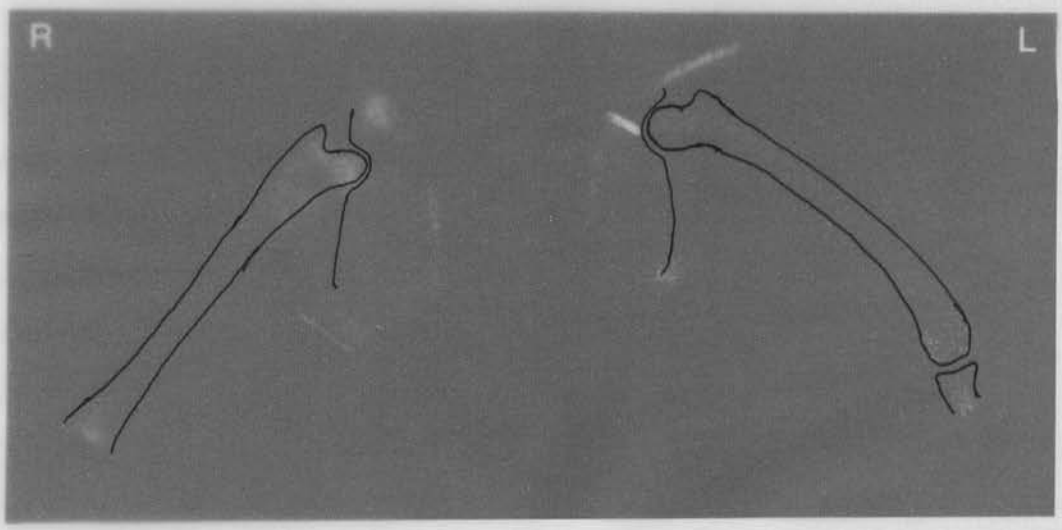
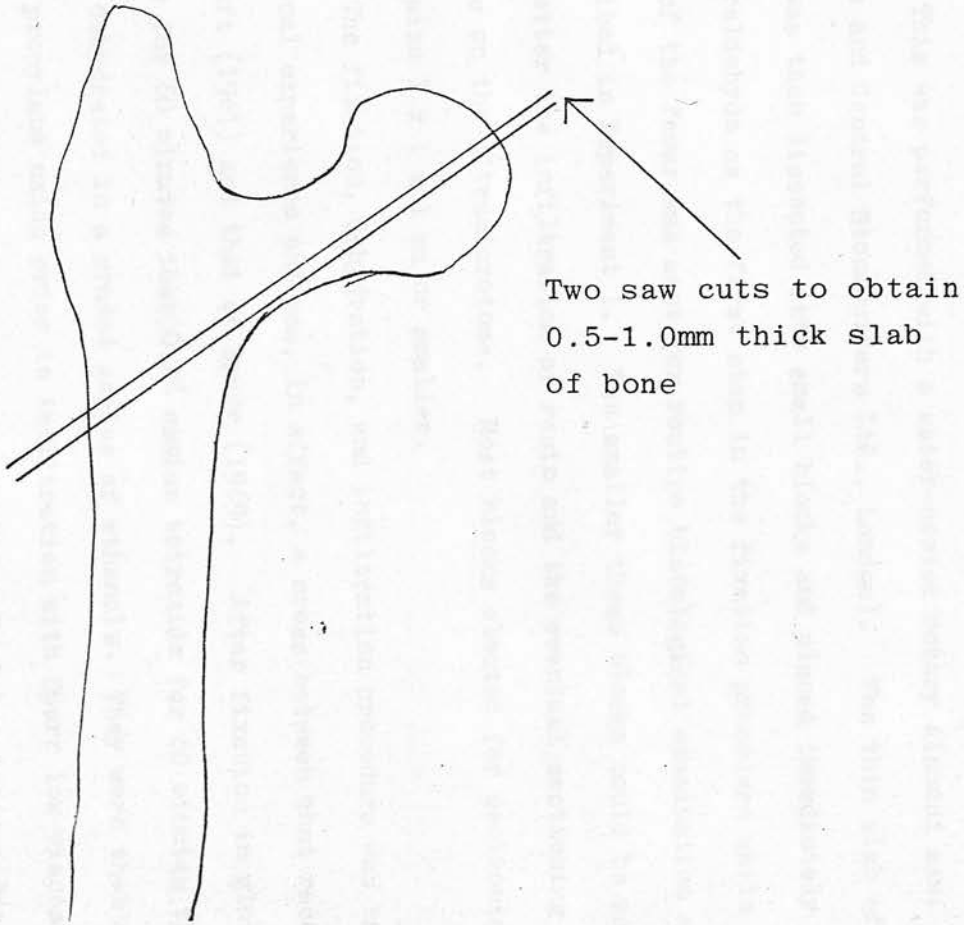


Fig. 33. Typical scintigraph showing increased uptake of radioisotope in right proximal femur.

Fig. 32. Typical scintigraph showing increased uptake of radioisotope in right proximal femur (see text).

Fig. 33. Typical scintigraph showing increased uptake of radioisotope in right proximal femur.

Fig. 34 Plane of section of proximal rabbit femur



8 to 15 weeks after the original operation. After death, the aim was to obtain small blocks of cancellous bone from the area of positive scintigraphy as rapidly as possible to start the embedding process for the Spurr resin. The right femur was dissected out and two parallel saw cuts made in a coronal plane passing obliquely through the head and neck of the proximal femur as shown on the longitudinal diagram in Fig. 34. This was performed with a water-cooled rotary diamond saw (Agate and General Stonecutters Ltd., London). The thin slab of bone was then dissected into small blocks and placed immediately into glutaraldehyde as the first step in the fixation procedure while the rest of the femur was sent for routine histological examination as described in Experiment 1. The smaller these blocks could be made the better the infiltration of resin and the eventual sectioning properties on the ultramicrotome. Most blocks elected for sectioning were of a size 1 x 1 x 1 mm or smaller.

The fixation, dehydration, and infiltration procedure was based on local experience and was, in effect, a cross between that recommended by Luft (1961) and that of Spurr (1969). After fixation in glutaraldehyde for 60 minutes then 0.2% osmium tetroxide for 60 minutes the blocks were dehydrated in a graded series of ethanols. They were then changed into propylene oxide prior to infiltration with Spurr low viscosity epoxy resin (constituents supplied by T.A.A.B. laboratories, Reading, England). This was done as it had been found to allow more rapid infiltration - possible because of the lower boiling point of propylene oxide. Fixation, dehydration and infiltration were performed at room temperature. After infiltration with 50% propylene oxide / 50% resin and one change in 100% resin the blocks were embedded in resin in polythene capsules (T.A.A.B. Laboratories) in an oven set at 60°C. The loss of radioactivity during this preparation is about

Table 15 Summary of Time Sequence

Injection ^{99m}Tc MDP			-1 to 3 h
Animal sacrificed, (R) femur dissected out, proximal femur cut with water-cooled rotary diamond saw			0 h
Fixation (room temp)	Glutaraldehyde	60 min	
	Osmium tetroxide	60 min	
Dehydration (room temp)	70% ethanol	15 min	
	90% "	15 min	
	95% "	15 min	
	100% "	15 min	
	100% "	15 min	
	Propylene oxide	15 min	
Infiltration	Propylene oxide + 50% resin	30 min	
	100% Spurr resin	120 min	
		6 h	6 h
Embedding (oven 60°C) minimum 8 h		overnight	
Sectioning Porter-Blum ultramicrotome.			22-23 h
Application of K5 nuclear emulsion.			24 h

60% (Heyes, 1979). The time course of the procedure is summarised in Table 15. It is the same total of 14 hours as achieved by Tilden and co-workers but we were not able to use this short time without working throughout the night. An overnight step had to be incorporated at some stage and we elected that this should be during embedding as this had to occupy 8 hours anyway.

After this overnight embedding the blocks were sectioned on an ultramicrotome. The microtome used was a Porter-Blum MT1 and sections 1 - 2 µm thick were cut with a glass knife. We attempted to use other ultramicrotomes without success, and our experience appears to agree with that of other workers (Table 14). The sections were floated on to distilled water or phosphate buffer, though we did not anticipate significant loss of phosphate into the distilled water (as described by Boothroyd, 1964) with sections of this thickness. They were transferred to microscope slides which had been cleaned with an acid solution of potassium bichromite (100g. potassium bichromate in 850 ml. water plus 100 ml. concentrated sulphuric acid) and subbed with a filtered solution of gelatin (gelatin 5.0g and chrome alum ($K_2SO_4.Cr_2(SO_4)_3.24H_2O$) 0.5g per 1. of water) and gently evaporated to dryness.

The autoradiography was performed very much as recommended by Rogers (1967). The available darkroom was emptied and thoroughly cleaned and all equipment not required for the autoradiography stored in another room. The room was recleaned before each use to ensure a dust-free environment. As far as possible the room temperature was kept between 20 - 25°C. and the relative humidity within the range 40 - 45%. This was not fully possible on hot summer days as the cooling system was inadequate in these circumstances (see Table 16). The darkroom was illuminated by a single 15w bulb behind a No. 1 filter (Kodak Ltd.) mounted 1m. above the bench surface. Ilford K5 nuclear tracking

Table 16. DARKROOM CONDITIONS DURING AUTORADIOGRAPHY

DATE	BEFORE AUTORADIOGRAPHY					AFTER AUTORADIOGRAPHY				
	DRY BULB TEMP. °C.	WET BULB TEMP. °C.	WET BULB DEPRESSION	DEW POINT °C.	RELATIVE HUMIDITY %	DRY BULB TEMP. °C.	WET BULB TEMP. °C.	WET BULB DEPRESSION	DEW POINT °C.	RELATIVE HUMIDITY %
30 MAR.	21.5	14.5	7.0	8.3	42.5	21.5	15.0	6.5	9.5	46
5 APR.	22.5	15.0	7.5	8.5	40.5	22.5	16.1	6.4	11.0	48
10 MAY.	23.0	16.7	6.3	11.4	48	24.4	17.5	6.9	12.5	47.5
13 JUN.	23.1	15.6	7.5	9.3	41.5	23.3	15.8	7.5	9.6	42.0
26 JUL.	25.0	16.6	8.4	9.9	38.5	25.4	16.6	8.8	9.6	37.0
16 AUG.	25.0	18.6	6.4	14.3	51.5	26.1	19.2	6.9	14.7	49.5

emulsion (Ilford Ltd., Basildon, England) was used. All glassware was cleaned with an acid solution of potassium bichromate and well rinsed with distilled water and this was repeated before each use. A cover with holes in which to place two measuring cylinders and to suspend a dipping jar was made for a water bath. The temperature of the water bath throughout the procedure was 42°C. The emulsion was prepared in darkness with safelight illumination only and the dipping and drying of autoradiographs carried out under the same conditions. More than 25 mls. of emulsion was transferred to a 50 ml. measuring cylinder (with a black paint line at 25 ml.) with print forceps and melted in the water bath (10 minutes). An accurate 25 ml. quantity was measured using a second measuring cylinder (again with a black paint line at 25 ml.) and the quantity gently poured into 25 ml. of 2% glycerol solution in the dipping jar.

After two minutes a trial subbed slide was dipped in the emulsion and held vertically up to the safelight to ensure that there was even distribution of emulsion over the centre of the slide and that no bubbles were present. If bubbles were seen the emulsion was left for a further two minutes and a trial dip repeated. The specimen slides were then dipped and held vertically while excess emulsion and the emulsion on the back of the slide were wiped off with Kleenex medical wipes. They were then placed on a cooled metal plate for 45 minutes and dried with gentle circulation of air produced by a hair dryer diagonally mounted 2m. away. The rationale of using a cooled metal plate is to gel the emulsion before drying takes place (Rogers, 1967). The slides were then placed in light-tight plastic slide boxes and exposed overnight in a refrigerator (at 4°C) not containing any radioactive material and not near any known source of

radioactivity. Two control slides were included with each set of specimen slides. One was a slide known to be non-radioactive (prepared from a rabbit not given ^{99m}Tc diphosphonate before sacrifice) to guard against false positive results and the other a slide deliberately exposed to light to develop the emulsion and check that there was an even layer over the specimen. The exposure time was chosen after reviewing the other autoradiographic experience with ^{99m}Tc summarised in Table 13. Because of the 6.1 hour half-life of ^{99m}Tc there seemed very little to be gained by long exposure. The time chosen was about 22 hours.

The next morning development and fixation was performed in the same darkroom using the same lighting conditions. Development and fixation were performed with the slides horizontal to avoid displacement of the emulsion. Development was in D-19 developer (Kodak Ltd., Manchester, England) for 3 minutes. The slides were then rinsed in distilled water for 10 seconds, fixed in 25% thiosulphate solution for 3 minutes, well rinsed, and allowed to dry in a dust-free place. These optimum times were arrived at by varying the time exposures within a single batch of slides. The stains compatible with dipping autoradiography are rather limited (Belanger, 1961; Thurston and Joftes, 1963) and the autoradiographs were post-stained through the overlying photographic emulsion with toluidine blue and cover slips applied for microscopic examination. The sections were examined on a Nikon microscope by transmitted light, and with incident darkground illumination.

As it proved impossible to maintain any particular orientation of the blocks during embedding and the toluidine blue was found unreliable in differentially staining immature bone mineral, two rabbits

selected for autoradiographic study were given 150 mg.oxytetracycline intramascularly 5 days before sacrifice. Some of the sections from the ultramicrotome in these two rabbits were mounted directly and examined by ultraviolet light (they were not exposed to autoradiographic emulsion or post-staining with toluidine blue in case either of these removed the oxytetracycline). One of these two rabbits had sections prepared from an area <u>not</u> showing increased uptake of ^{99m}Tc diphosphonate on scintigraphy.

RESULTS.

The preparation technique employed for the autoradiography produced entirely satisfactory thin sections of undecalcified cancellous bone. The excellent cellular and mineral detail can be seen by reference to Fig. 35. This technique should be both easily reproducible and generally applicable as equipment available in many research laboratories was used. The Spurr resin constituents are readily available as a ready-prepared kit and the thin sections were cut with glass knives made in the laboratory (LKB knifemaker), not with expensive diamond or tungsten carbide blades. The 1 - 2 µm sections obtained should not only be useful for autoradiographic studies but should be eminently suitable for electronmicroscopy.

The autoradiographic technique took several attempts before useful results were obtained. Specimen autoradiographs are reproduced in Figs. 36 to 40. These are from a rabbit sacrificed two hours after intravenous administration of ^{99m} Tc methylene diphosphonate (MDP). The developed silver grains are found in relation to bone mineral (see the low-power view, Fig. 36, where the mineral has a characteristic

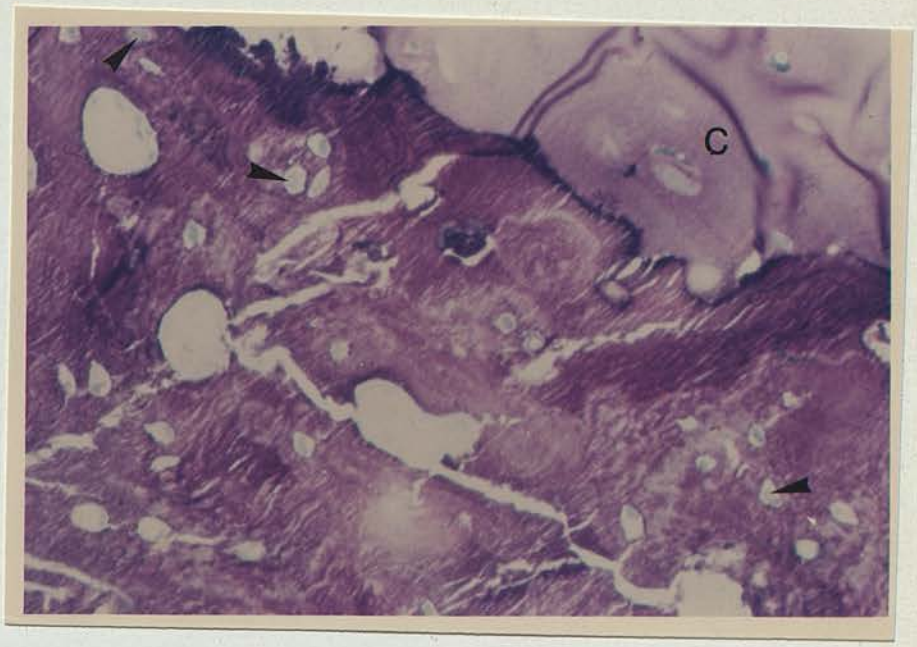


Fig. 35 Cellular and mineral detail in subchondral bone. Note cartilage (c) at top right and osteocytes (some arrowed). Toluidine blue x50

Fig. 36 Autoradiograph obtained Toluidine blue. Low power view showing radioactivity localised to bone mineral. x420.

"herring-bone" appearance and the clear areas are marrow spaces. All the autoradiographs are only lightly poststained with toluidine blue to allow easier identification of developed silver grains. Higher magnification (Figs. 37 - 40) shows that many of the groups of developed silver grains are closely related to cell spaces within the bone. With sections only 1 - 2 μm thick, cells are only seen in cross-section and this may not include the cell nucleus. Some cell spaces appear empty even when radioactivity is localised to the neighbouring mineral but this is probably because the thin section did not pass through the cell (Figs. 37, 38). I was surprised that even in autoradiographs of a rabbit sacrificed 2 hours after administration of labelled diphosphonate some of the radioactivity was still detected as being localised to the cytoplasm of the osteocytes (Figs. 39, 40). Histological sections of adjacent cortex showed necrosis with endosteal new bone formation (Fig. 41), but even more convincing evidence that sections were obtained from an area of new bone formation was gained from examination of adjacent sections (cut from the same blocks) by ultraviolet light. These showed signs of tetracycline fluorescence around many lacunae (Fig. 42), whereas in a control rabbit with no bone necrosis in the proximal femur (and no increased uptake on scintigraphy in this site) only very occasional tetracycline fluorescence was seen (Fig. 43). This confirmed that the area was one actively laying down new bone mineral in the five days before the rabbit was killed. Autoradiography in the control rabbit was entirely negative and routine histological examination did not show any abnormality in the proximal femur.

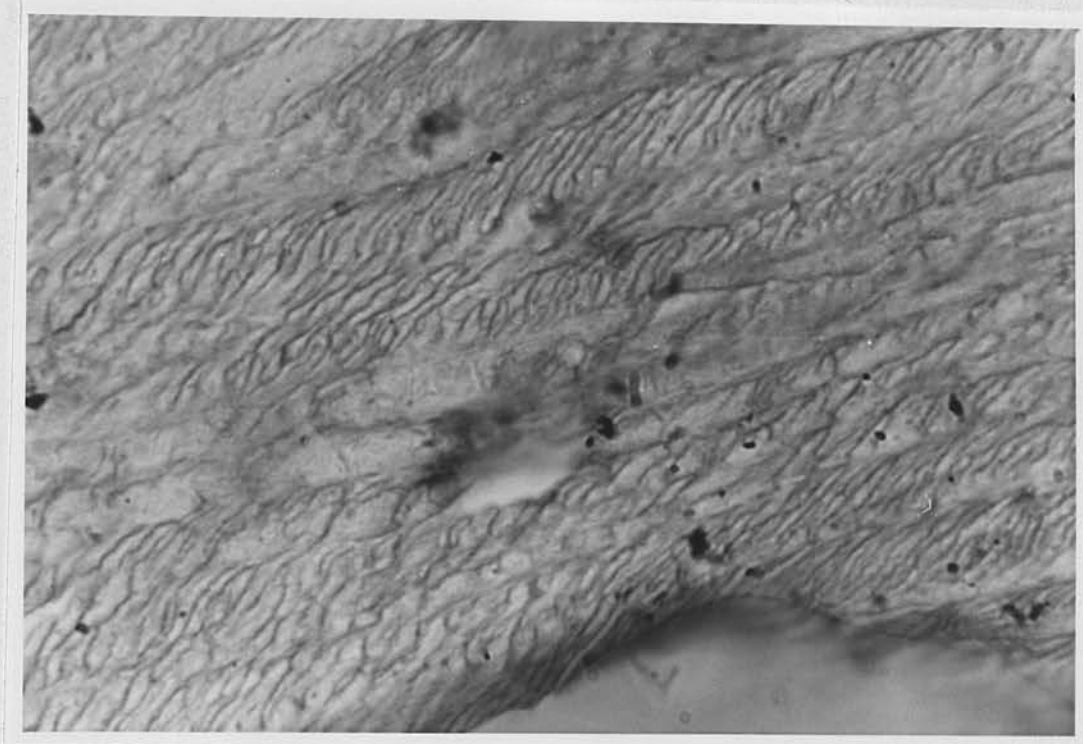


Fig. 37. Autoradiograph stained toluidine blue. Note localisation of radioactivity to bone mineral surfaces. x 1250.

Fig. 38. Autoradiograph stained toluidine blue. Note localisation of radioactivity to the bone mineral surface. x 1250

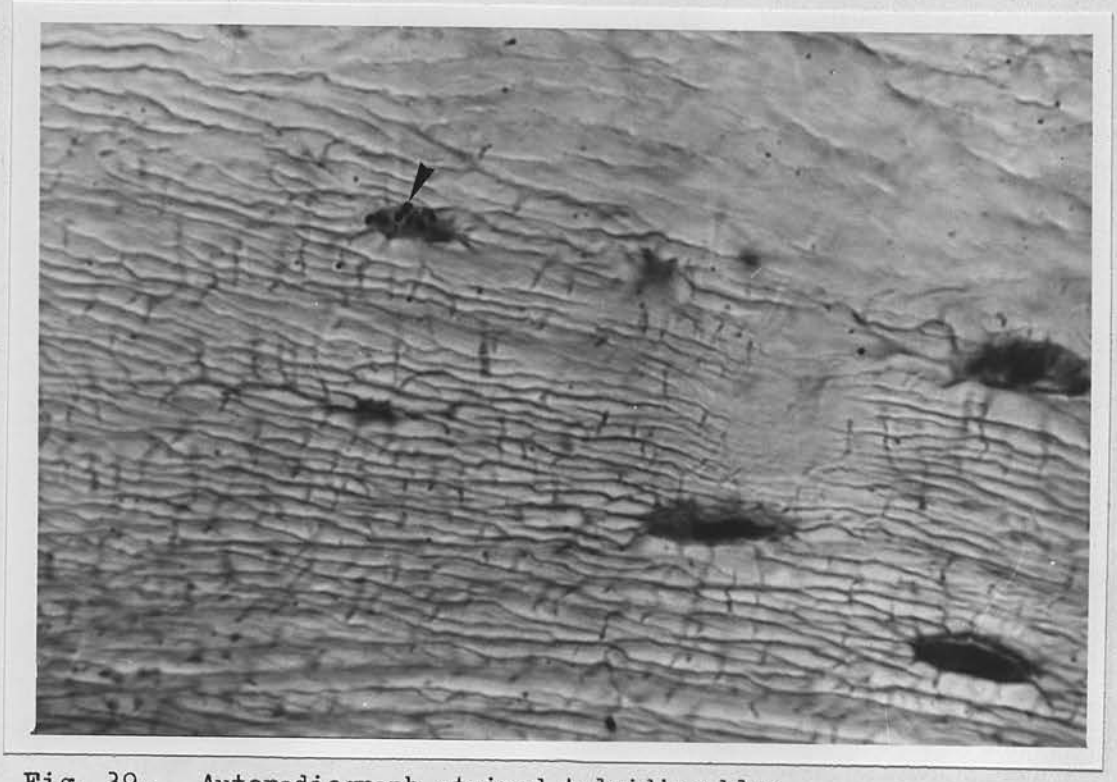


Fig. 39. Autoradiograph stained toluidine blue. Radioactivity (arrowed) localised to osteocyte cytoplasm. x 1250

Fig. 40. Autoradiograph stained toluidine blue. Radioactivity (arrowed) localised to osteocyte cytoplasm. x 1250.

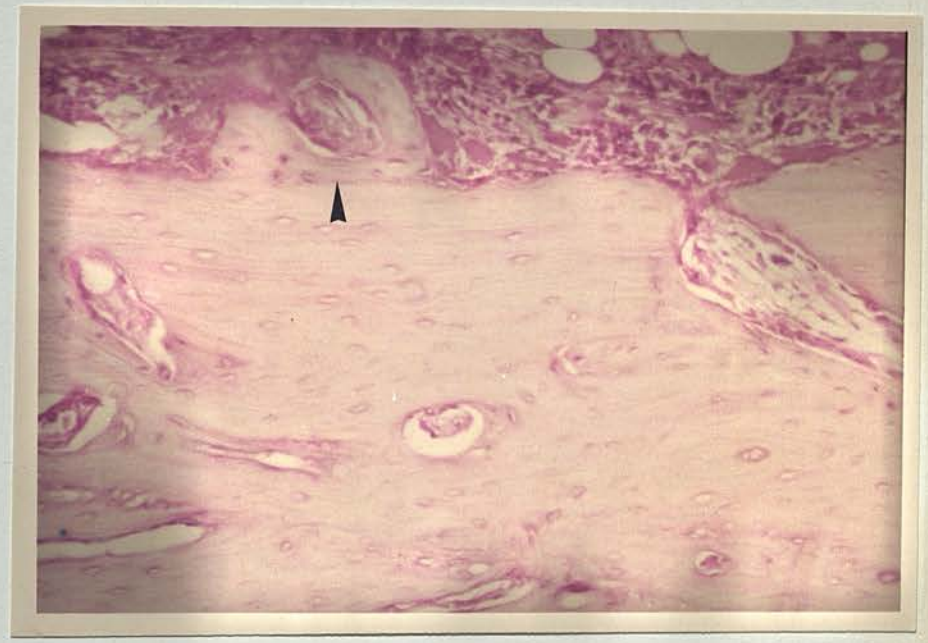


Fig. 41. Histological section of adjacent cortex of proximal femoral shaft of the same rabbit studied autoradiographically in Figs. 36 - 40. Note empty lacunae in cortex and endosteal new bone formation (arrow). x 50.

Fig. 42. Tetracycline fluorescence around lacunae in same area of proximal right femur of the rabbit studied autoradiographically in Figs. 36 - 40. U.V. light x 800.

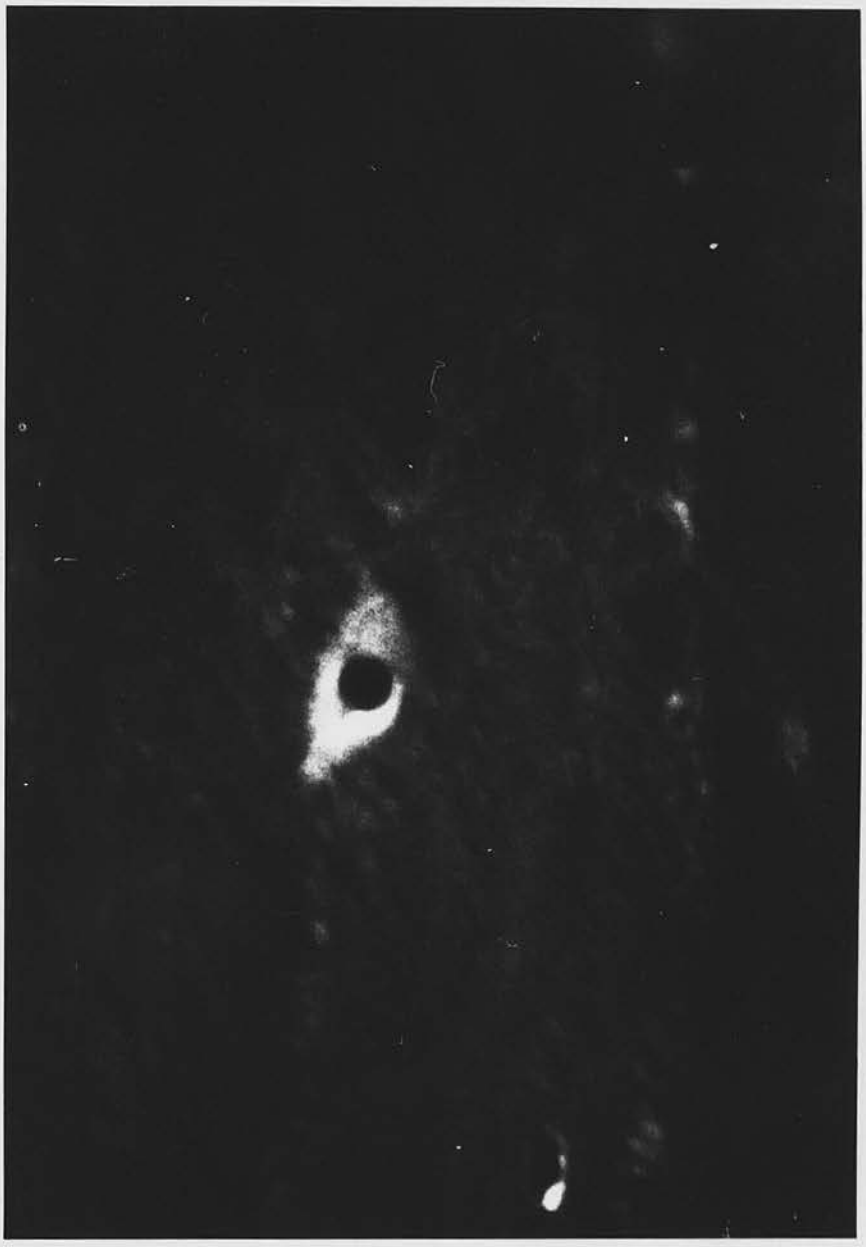


Fig. 43. Tetracycline fluorescence around very few lacunae in proximal right femur of a rabbit with no artifically induced bone necrosis. U.V. light x 800.

DISCUSSION.

The technical problems of developing a method for rapidly embedding and sectioning undecalcified bone to a thickness of 1 - 2 μm suitable for autoradiographic and electronmicroscopic studies appears to have been successfully overcome. Since preliminary reports (Stothard and Walder 1978a, 1978b), another paper has been published using a very similar preparation technique (Greiff, 1978) for the microautoradiographic study of fracture healing. Epon-Araldite was used for embedding rather than Spurr resin, but the other technical details appear to show a solution to the problems outlined in the hypothesis above along the lines described in this experiment. His autoradiographic technique is indistinguishable from that outlined above from the information provided. It therefore appears that this approach can be recommended as providing a reproducible technique in more than one author's experience.

The results of the autoradiographic studies are of interest and indicate that the osteocytes may have a role in the increased uptake of bone-seeking radionuclides found in areas of new bone formation. This supports the work of Nichols and Rogers (1972) and Matthews et al. (1973) which showed that bone cells could be made to exchange calcium and phosphate with the surrounding extracellular fluid. Other autoradiographic studies have differed in their results. Tilden et al. (1973), in autoradiographs performed on specimens removed 3 hours after administration of labelled polyphosphate, shows examples of radioactivity in rings of radius 10 - 30 μm around osteocyte lacunae whereas Greiff (1978) shows examples of radioactivity diffusely scattered within mineralised bone with no accumulation around the osteocytes.

There has been much discussion about a possible cellular role in calcification (Eanes and Posner, 1970) but less about a cellular role in determining the localisation of skeletal imaging agents. This may be because *in vitro* experiments show affinity of these agents for hydroxyapatite in the absence of bone cells (Jung *et al.*, 1973)and it is therefore assumed that this happens *in vivo*. This has led to statements that the labelled diphosphonate "chemisorbs at kink and dislocation sites on the surface of apatite" (Jones *et al.*, 1976) and that "the increased isotope activity seen on a bone scan after a fracture is primarily related to an increase in bone blood supply" (Hughes, 1977).

At the time this experiment was performed, existing reports of the localisation of ^{99m}Tc labelled polyphosphate in bone by micro-autoradiography had concentrated on the time interval of 3 - 4 hours after isotope administration. This was helpful in confirming that the isotopes eventually localised within bone mineral, but did not explain why areas with most mineral, e.g. the cortex of long bones, show very little isotope uptake on routine scintigraphy. Examination at this time interval was probably not going to aid our understanding of the mechanisms involved in causing the areas of localised increase of isotope uptake seen in a diverse range of skeletal anomalies and pathological processes.

Since the experiment was performed, there have been two reports of microautoradiographic studies concentrating on time intervals one hour after intravenous administration of isotope Greiff (1978) used ^{99m}Tc-polyphosphate in studying rat tibial fracture callus and epiphyseal growth plates. Radioactivity was found to be diffusely scattered within the bone mineral, with no accumulation around

osteocytes. Khan et al. (1979) used ^{99m}Tc- methylene disphosphonate in a similar study of rat epiphyseal growth plates. Radioactivity was found along the Haversian systems and coating the trabeculae of bone but was not present in established bone.

This disagreement means that further studies are necessary. Khan et al. (1979) speculate on a "factor dependent on the nature of the bone matrix" as governing the incorporation of isotope into bone, but the only evidence for implicating the bone matrix rather than, for example, the bone cells appears to be the localisation of radioactivity to the surface of bone trabeculae. There is good autoradiographic evidence for a cellular role in the formation of bone matrix (Carneiro and Leblond, 1959), and the present study indicates that the cells may also have a role in the concentration of skeletal imaging agents in areas of new bone formation.

CONCLUSIONS.

A technique is described for the rapid preparation of thin sections of undecalcified cancellous bone with a surface smooth enough for autoradiography.

Some of the early results of autoradiography are presented. They indicate that the enhanced concentration of skeletal imaging agents in areas of new bone formation may be a result of an increase in their uptake by the bone cells.

EXPERIMENT 9 : SKELETAL SCINTIGRAPHY IN MAN.

HYPOTHESIS.

Skeletal scintigraphy is a sensitive technique which can detect many osseous abnormalities. It is not at all specific and it is at present thought that the technetium - 99m phosphate compounds "are actively absorbed on to the surface of newly formed hydroxyapatite crystals and it has been shown that the rate of uptake and the concentration of these materials is related to two factors:
1. The rate of production of new hydroxyapatite crystals, i.e. osteoblastic activity; and 2. The blood flow to the area." (Maisey, 1978). An area with one or both of these factors for any reason will be visualised as an area of increased uptake, or 'hot spot', as discussed in Chapter 3.

There have been many instances of caisson disease of bone showing such positive scintigraphic appearances (Cox, 1974; Gorten and Cooley, 1974, Gregg, 1977) but there is at present no published evidence that the investigation of at risk subjects will lead to the earlier detection of caisson disease of bone. Gregg et al. (1977a) compared skeletal scintigraphy and radiography in rabbits with experimentally-induced osteonecrosis and found that earlier localisation of lesions was possible by scintigraphy than by radiography. The usefulness of skeletal scintigraphy in the early detection of experimental osteonecrosis in rabbits has been confirmed in Experiment 7.

Gregg (1977) studied skeletal scintigraphy in a group of men with caisson disease of bone (seen on radiographs) who had not worked in compressed air for at least ten years. After ten years the lesions might be considered static, and all except four lesions had not visibly changed on radiographic examination during this time. Despite this,

some had an increased uptake of skeletal imaging agent. Scintigraphy also provided some false positives (i.e. areas which on radiography appeared normal) and it seemed unlikely that these areas represented any abnormality related to the original hyperbaric exposure. However, Lotke et al. (1977) studied osteonecrosis of the knee with scintigraphy and discovered several patients with a typical history and scintigraphic appearance whose radiographic examination always remained normal. They felt that osteonecrotic lesions were probably occurring in these patients but that they were too small to be demonstrated radiographically and that they recovered spontaneously.

Many 'normal' anatomical variants and benign bone lesions show an increased uptake of skeletal imaging agents (O'Mara and Baker, 1973). Several of these lesions, including areas of osteonecrosis, have also been reported as occasionally showing a decreased uptake of skeletal imaging agents producing a "cold spot" on scintigraphy (Goergen et al., 1974). A potential problem for using skeletal scintigraphy as a screening method for early diagnosis of dysbaric osteonecrosis is that its non-specificity would give rise to an unacceptable number of false positive results. It seemed necessary to set up a formal survey group of men at risk who would undergo scintigraphy at regular intervals. Permission was obtained from the Medical Research Council Radiation Protection Committee (subsequently called the Isotope Advisory Panel of the Department of Health and Social Security) for annual examination of a group of 25 men, using a dose of 10 mCi of ^{99m}Tc instead of the usual clinically used dose of 15mCi. The survey group was planned to consist of men with no previous exposure to increased ambient pressure who were starting careers as commercial divers. So many of these men had done some previous sports diving that it was not possible

to exclude this from entrants into the survey group, but all men with a history of having commercial diving experience or of having worked in compressed air on civil engineering contracts were excluded.

It would be hoped that all these scintigraphs would be normal initially, so that any 'hot spots' seen in subsequent years might be interpreted as possible caisson disease of bone. To aid in interpreting the likely appearance of 'hot spots' related to caisson disease of bone scintigraphy was also performed on a number of men with definite or doubtful radiological appearances of caisson disease of bone.

MATERIALS AND METHODS.

The survey group contained 25 men aged 20-30 referred by the Government Training Services Agency to Newcastle-upon-Tyne for medical examination under the Department of Energy arrangements for a certificate of fitness for commercial diving. Prior to medical examination these applicants had been selected by interview and psychological testing as being suitable for training to become divers. The medical examinations were all performed in the Department of Industrial Health of the University of Newcastle-upon-Tyne. Out of the four or five men being examined each appropriate morning, two were selected for scintigraphy. The selection was arbitrary in that the two men invited to volunteer were those with addresses nearest to Newcastle-upon-Tyne (This was to aid follow-up in future years as it seemed more likely that these men would seek to have their future annual medical examinations for a certificate of fitness to dive performed in Newcastle-upon-Tyne). Every man invited to volunteer for scintigraphy agreed to do so, and I am very grateful to them for this. The majority of the medical examinations were performed by me, as one of the three

doctors at that time in Newcastle-upon-Tyne authorised by the Department of Energy to issue certificates of fitness to dive. All the injections of skeletal imaging agent were performed by me at the time of routine haematocrit and sickledex estimation as required by the medical examination. The dose used was 10m Ci of ^{99m}Tc attached to either commercial Osteoscan(1-hydroxyethylidene 1, 1-diphosphonate or EHDP) or commercial Osteolite (methylene diphosphonate or MDP). These were prepared and labelled by the Medical Physics Department of the University of Newcastle-upon-Tyne. A minimum of three hours later each man had scintigraphy performed at the Regional Medical Physics Centre using an Ohio Nuclear Series 100 gamma camera. All the scintigraphs were performed under my personal supervision to ensure a standard technique. Initially a high sensitivity parallel collimator was used but the time taken for the required examination with this collimator was unacceptably long and a converging collimator was used for all except the first two examinations. This gave just as good definition but a rather smaller field of view. It greatly reduced the time taken for each examination. The procedure adopted was as follows. The man first voided, being requested to be careful to avoid urine contamination of his clothing, and then lay supine on the examination trolley.

The scintigraphs of individual joints were performed in the following order: Anterior view of right hip and proximal femur, corresponding view of left side, Anterior view of right shoulder and proximal humerus, corresponding view of left side, Anterior view of right knee and distal femur, corresponding view of left side.

The right hip scintigraph was performed with a 5^{o} lateral tilt of the collimator to exclude as much of the radioactivity in the bladder as possible. A pre-set reading of 300,000 counts (300k)

was used and the time taken for this count noted. The left hip scintigraph was then performed with the opposite 5^{o} lateral tilt to give a comparable scintigraph to the right side. The time exposure for this view was pre-set to be the same as for the right hip and the reading of the number of counts noted.

Attention was then turned to the right shoulder and the collimator positioned as close to the anterior of the shoulder as possible. This was achieved in a standard manner by rotating the subject's head to the opposite side and tilting the collimator 5^{o} laterally and 10^{o} cephalad so that the right side of the chest and head were just NOT touching it. A pre-set reading of 200K was used and the time taken for this count noted. A comparable view of the left shoulder was then performed. As with the hip, the time for the joint on the left hand side was pre-set to be the same as for the joint on the right hand side and the reading of the number of counts noted.

The last joints on which scintigraphy was performed were the knees. The collimator was positioned horizontally as close as possible to the knee and with the field of view centered on the upper border of the patella with the limb in the neutral position of rotation. A pre-set reading of 120K was used and the time taken for this count noted. A comparable view of the left knee was then performed for the same time period (pre-set) and the reading of the number of counts noted.

Occasionally views of other bones were used in men referred with widespread osteonecrosis.

Using a converging collimator, the overall gamma camera time required for the six standard views was usually between 35 - 50 minutes.

This same protocol was also used for scintigraphy of men referred to the author specifically for this examination because they had

developed symptoms suggestive of osteonecrosis affecting the hip or shoulder joint or to aid interpretation of radiographs showing areas of suspected osteonecrosis. It was felt that examination of a file of positive scintigraphs associated with proven dysbaric osteonecrosis would be of help in the interpretation of abnormalities arising in the scintigraphs of the survey group in future years.

Although the scintigraphs performed in the Regional Medical Physics Centre are routinely stored on discs for computer display for the purposes of this research the scintigraphs were stored on polaroid film (Polaroid Corporation, Cambridge, Mass.) This was to allow direct visual comparison of many scintigraphs.

RESULTS.

The results are reported in three sections:-

(a) The findings of the baseline scintigraphic examinations of 25 men before they started commercial diving. (b) A comparison of the two diphosphonate skeletal imaging agents used. (c) The findings in the miscellaneous group of men with suspected or definite dysbaric osteonecrosis.

a) FORMAL SURVEY GROUP OF 25 COMMERCIAL DIVERS.

This is a long-term project and only the findings of the baseline examinations, performed before the men started commercial diving, will be reported here. Of the 25 men, 4 had abnormalities on the initial scintigraphic examination.

1) Subject W.B.: This 27 year old man had previous sports diving experience (as had many of the men) to a depth of 16 m. Scintigraphy showed a peardrop shaped area of increased uptake of isotope in the proximal right femur (Fig. 44). This corresponded to an area of

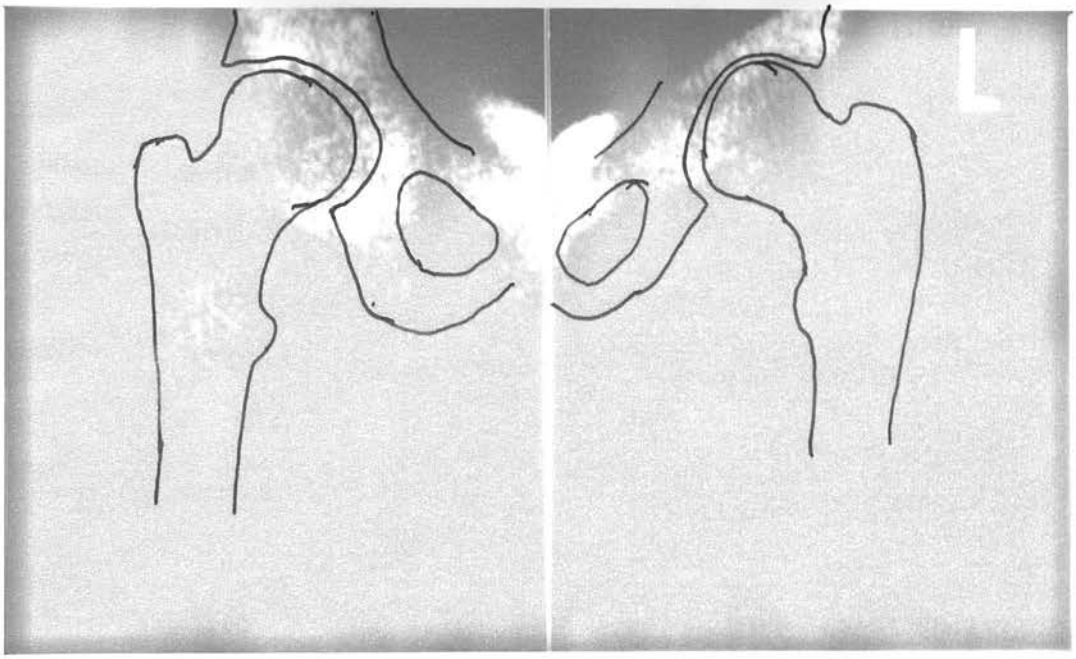


Fig. 44 Hip scintigraphs of subject W.B. Note the peardrop shaped area of increased isotope uptake in the proximal femur and the slightly greater uptake in the right femoral head compared with the left.

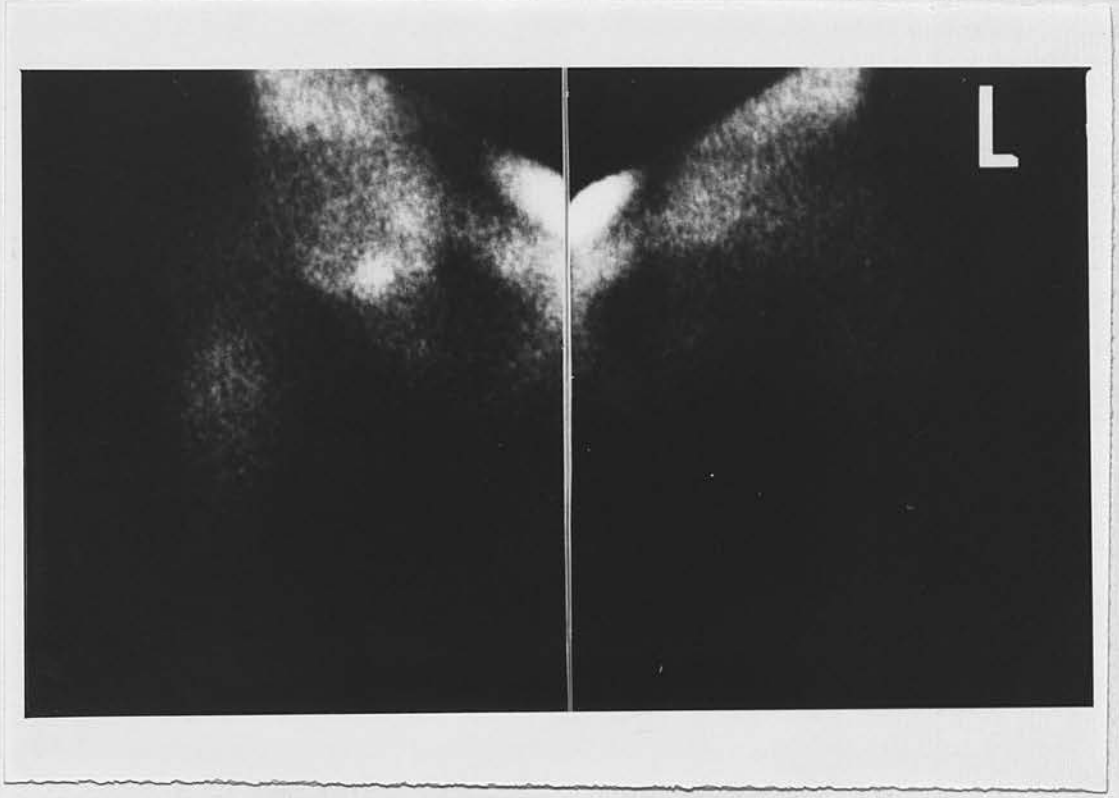


Fig. 44 Hip scintigraphs of subject W.B. Note the peardrop shaped area of increased isotope uptake in the proximal femur and the slightly greater uptake in the right femoral head compared with the left.

Fig. 45. AP Radiograph of Right hip of subjects W.B. (for comparison with Fig. 44).

Fig. 46. The same lesion as Fig. 45 three months later.

increased radiographic density (Fig. 45) Expert radiological advice was sought and the opinions expressed varied but the commonest was that the lesion was a benign 'enostosis' and none of the panel of experts who examine radiographs for the MRC decompression sickness central registry felt that this subject had caisson disease of bone. The radiographic appearance remained unchanged three months later (Fig. 46) and biopsy was undertaken. Sections from the biopsy specimen were kindly sent to the author and stained with hematoxylin and eosin in the same manner as already described for the animal bone specimens. Microscopic examination showed normal cortical bone throughout the sections.

2) Subject C.B. : This 29 year old man had a 'hot-spot' over the right distal femur on scintigraphic examination. Radiographic examination appeared normal and it was thought that this might represent urine contamination. However, scintigraphic examination the following year showed an unchanged appearance (Fig. 47) and this indicated that the 'hot-spot' was associated with a skeletal abnormality and not with chance contamination by urine. Radiographs on the second occasion were of better quality and showed a right bipartite patella at the site corresponding to the scintigraphic 'hot-spot'.

3) Subject T.A. : This 22 year old man exhibited a strongly positive 'hot-spot' over the medial compartment of the left knee (Fig. 48). The radiographic appearance was normal. He had no symptoms referable to his knee and clinical examination of his knee performed by me could elicit no abnormality. Specifically, he had not had a previous menis - cectomy.

4) Subject A.M. : This 27 year old man had two small spots of increased isotope uptake in the right axilla, in an area not over-

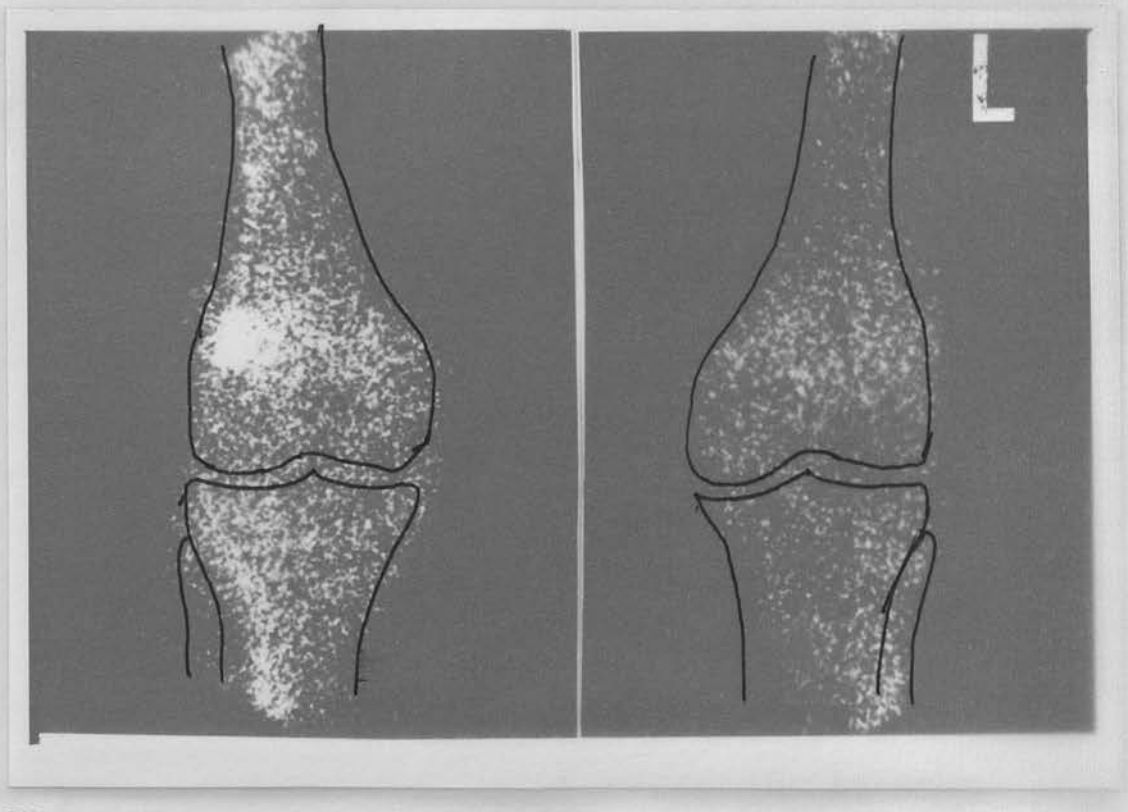


Fig. 47 Scintigraphs of distal femora of subject C.B. Note the 'hot-spot' on the right side, probably related to a bipartite patella.

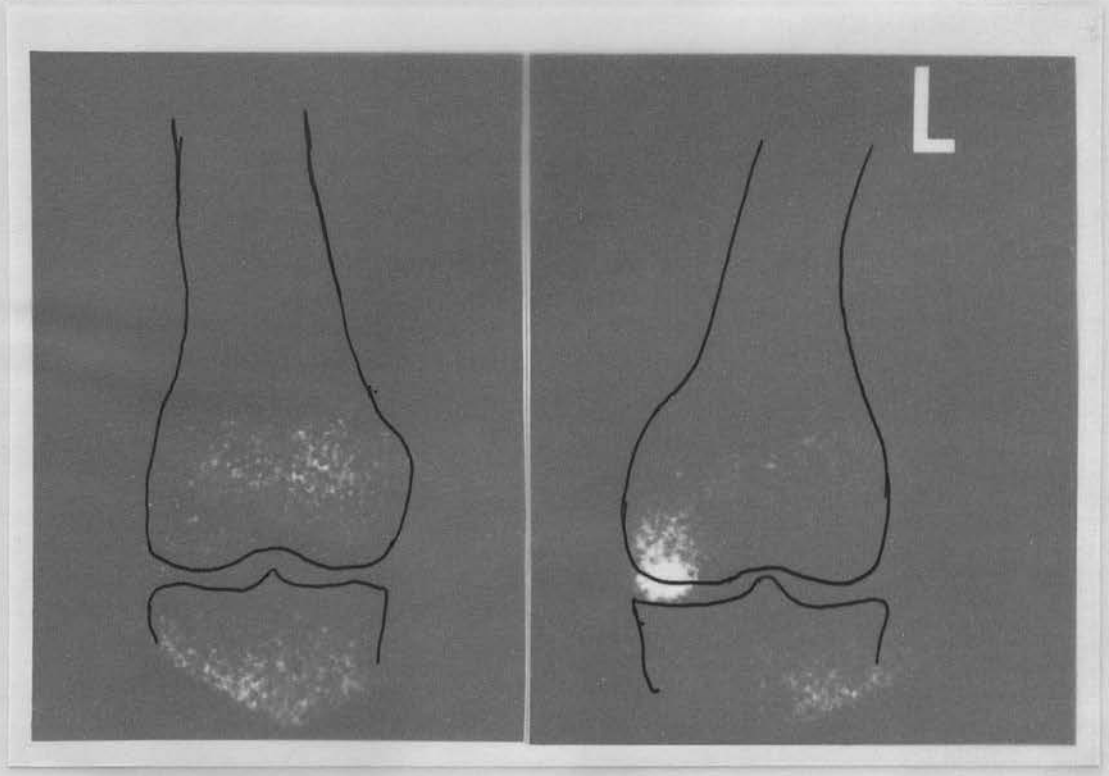


Fig. 48. Scintigraphs of distal femora of subject T.A. The 'hot-spot' over the medial compartment of the left knee is unexplained.

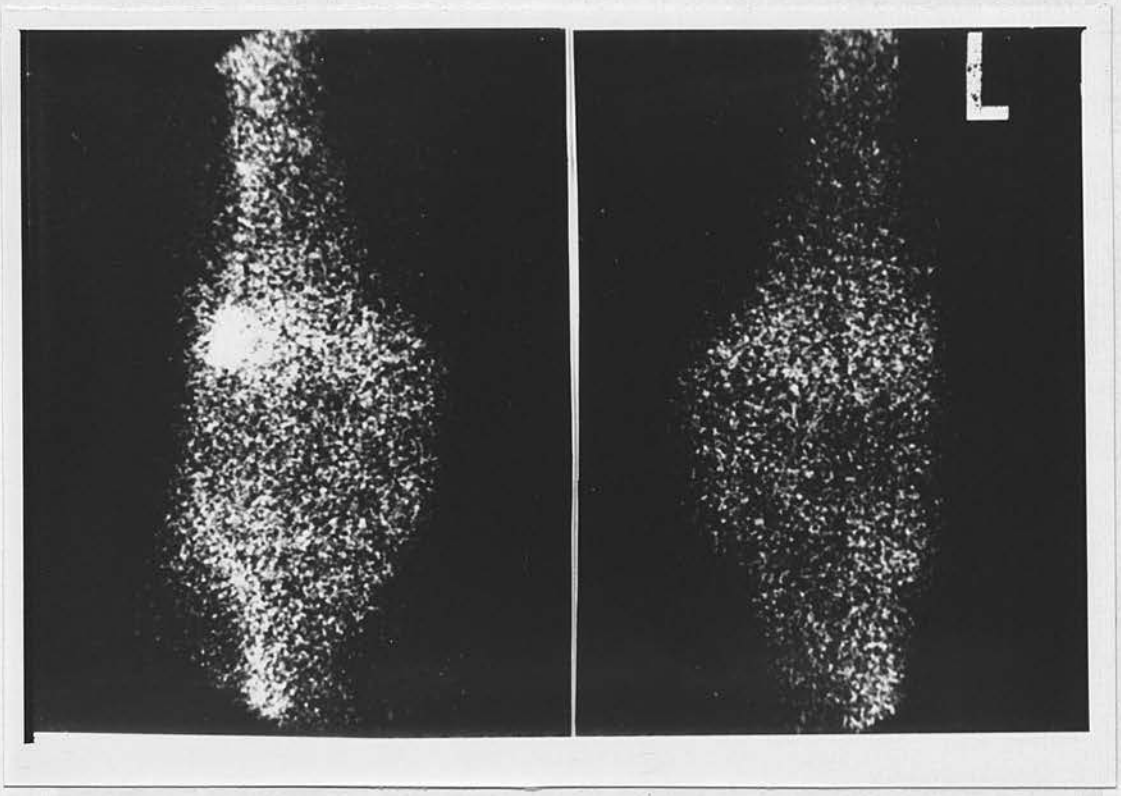


Fig. 47 Scintigraphs of distal femora of subject C.B. Note the 'hot-spot' on the right side, probably related to a bipartite patella.

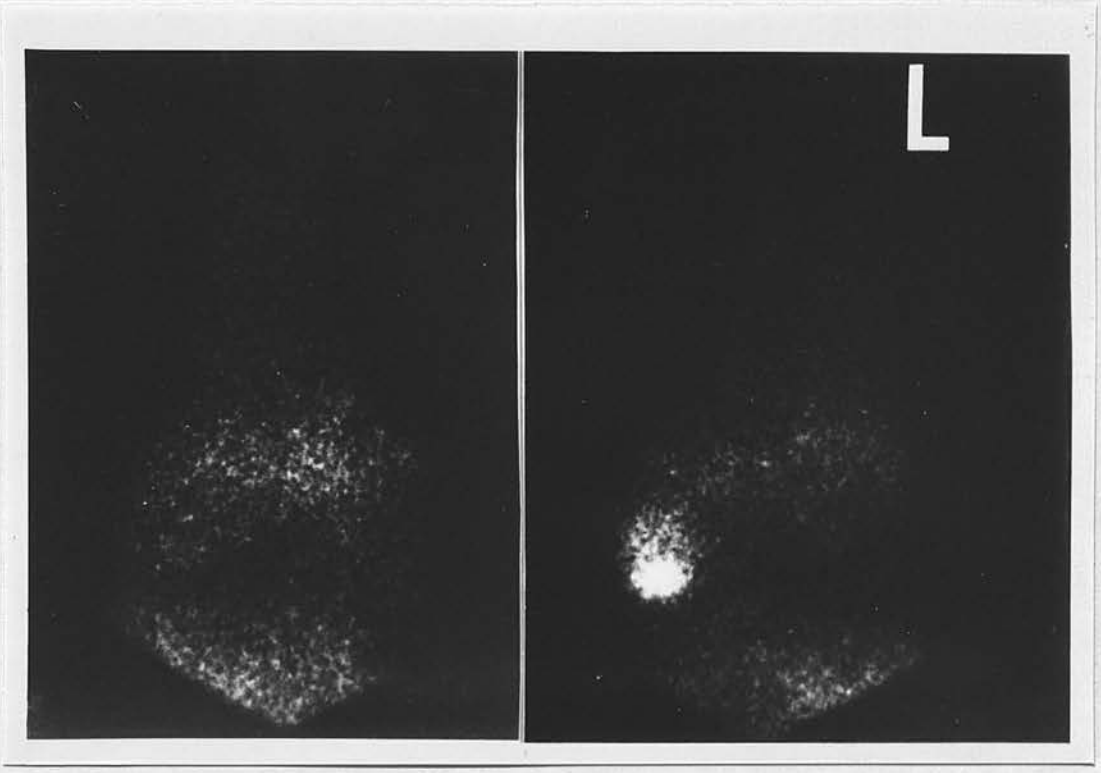


Fig. 48. Scintigraphs of distal femora of subject T.A. The 'hot-spot' over the medial compartment of the left knee is unexplained.

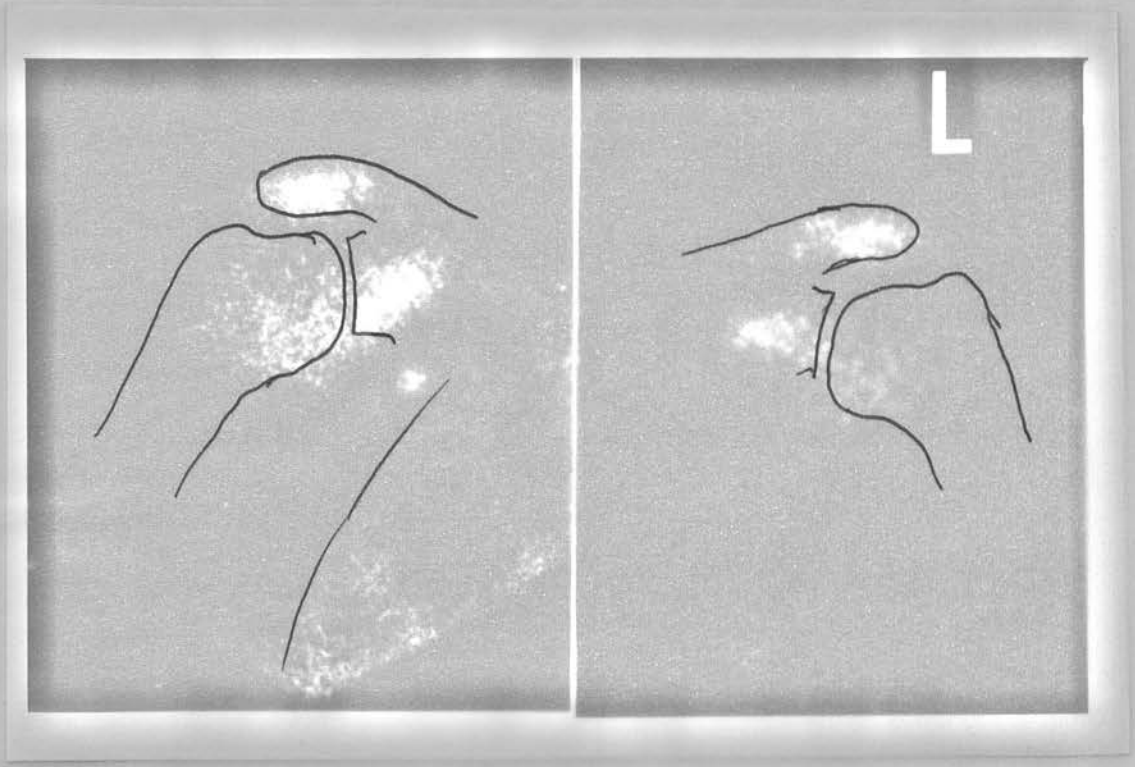


Fig. 49. Shoulder scintigraphs of subject A.M. Note the two small spots of increased isotope uptake in the right axilla.

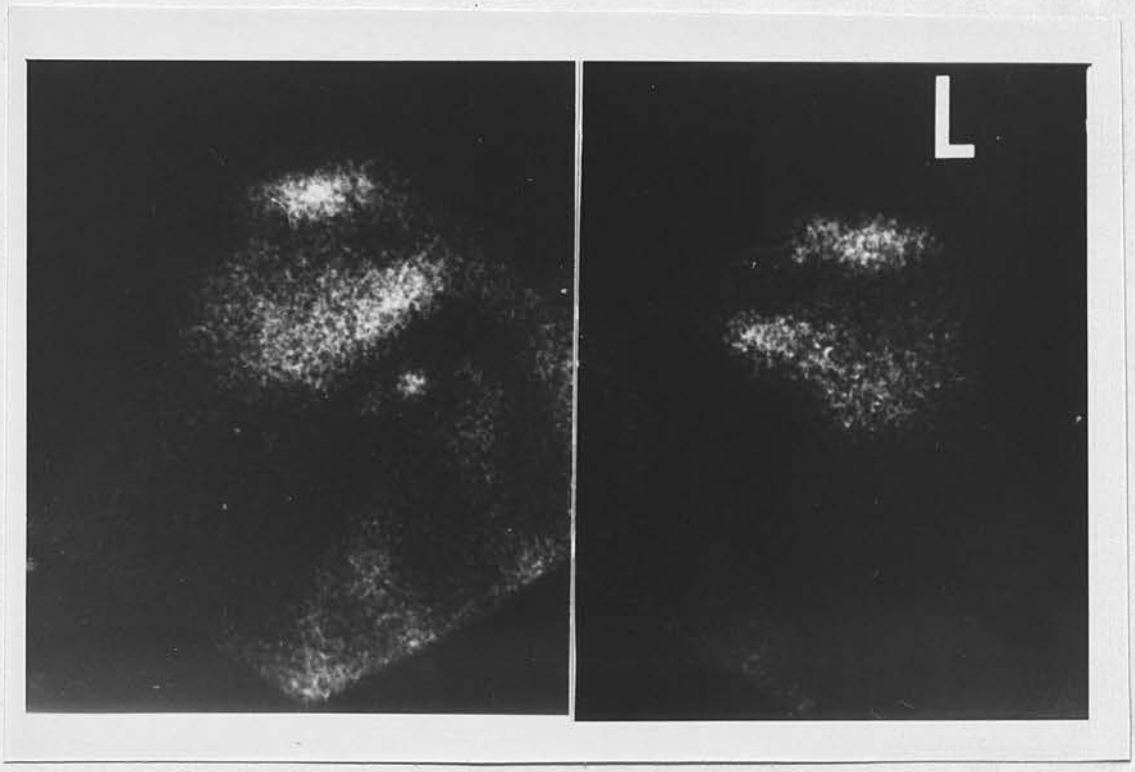


Fig. 49. Shoulder scintigraphs of subject A.M. Note the two small spots of increased isotope uptake in the right axilla.

lying bone at all. (Fig. 49). Radiographic examination was normal. The scintigraphic appearance remains unexplained and the annual follow-up examination is still awaited.

b) COMPARISON OF TWO DIPHOSPHONATE SKELETAL IMAGING AGENTS.

The choice of imaging agent was determined by the availability of kits EHDP (Osteoscan) or MDP (Osteolite) in the Medical Physics Department. MDP was steadily replacing EHDP for routine clinical whole-body scintigraphy over the period of study and when the formal survey group had been completed there were 10 subjects with scintigraphs using MDP and the remainder had scintigraphs using EHDP. The 10 MDP scintigraphs and the last 10 EHDP scintigraphs were made up into matched pairs A - J and submitted to two colleagues (J.W.H. and P.J.G.) for comparison. Neither knew which agent had been used for individual scintigraphs of any named diver. Each was asked to complete a form as shown on Fig. 50, grading the bone outline and blood background as unsatisfactory, satisfactory, or good; and deciding which set was superior. They were both also asked to give details on a separate form of all positive scintigraphs (i.e. those with abnormal areas of locally increased uptake of the radioactive tracer.)

The results are expressed in Tables 17 and 18. Table 17 shows that all the scintigraphs were technically satisfactory except that P.J.G. felt that the bone outline was poor on the hip films of one EHDP scintigraph. Similar numbers of EHDP and MDP scintigraphs were regarded as satisfactory or as good by each reader. Despite this, the more subjective assessment of which of each pair was the better set of scintigraphs showed a slight preference for MDP sets. The two readers agreed about 7 of the 10 matched pairs, and of these 4 were in favour of MDP and 2 in favour of EHDP and 1 pair no prefer-

DIVER SCINTIGRAPHS :			COMPARISON OF MATCHED PAIRS		
PAIR	FIRST DIVER IN PAIR		SECOND DIVER IN PAIR		BETTER SET (if equal write Nil)
	BONE OUTLINE *	BACKGROUND ACTIVITY *	BONE OUTLINE	BACKGROUND ACTIVITY	
A					
B					
C					
D					
E					
F					
G					
H					
I					
J					
	* Bone outline and Background activity : Code as 1 = Not satisfactory, 2 = Satisfactory, 3 = Good.				

Fig. 50. Form for completion for comparison of diver scintigraphs (see text).

TABLE 17.

Diver Scintigraphy

Comparison of MDP and EHDP by two observers (JWH & PJG)

Pair	MDP Scintigraph				EHDP Scintigraph				Better Set		
	Bone outline		Background		Bone outline		Background				
	JWH	PJG	JWH	PJG	JWH	PJG	JWH	PJG	MDP	NIL	EHDP
A	3	3	2	3	2	2	2	2	JWH PJG	-	-
B	2	3	2	3	2	2	2	2	JWH PJG	-	-
C	2	3	3	3	2	1	2	3	JWH PJG	-	-
D	3	3	3	3	3	2	2	2	JWH PJG	-	-
E	3	2	2	2	2	3	3	3	-	JWH	PJG
F	2	2	2	3	2	2	2	3	JWH	-	PJG
G	2	3	3	3	3	3	3	3	-	-	JWH PJG
H	2	2	2	3	2	2	2	3	-	JWH PJG	-
I	2	2	2	2	3	3	3	3	-	-	JWH PJG
J	2	3	3	2	2	2	3	3	-	JWH	PJG

TABLE 18.

ASSESSMENT OF ABNORMALITIES ON DIVER SCINTIGRAPHS

MAN	PJG ASSESSMENT	JWH ASSESSMENT.
E.W.	NORMAL	NORMAL
R.L.	"	"
L.M.	"	"
R.G.	"	"
A.W.	"	"
M.D.	"	"
T.H.	"	"
G.B.	"	"
J.W.	POS. RIGHT HUMERAL HEAD	POS. RIGHT HUMERAL HEAD
P.H.	NORMAL	NORMAL
M.S.	"	"
W.B.	POS. RIGHT PROX. FEMUR R. FEM HEAD	POS. RIGHT PROX. FEMUR and R. FEM. HEAD.
D.C.	NORMAL	NORMAL
T.A.	POS. MED FEMORAL CONDYLE LEFT	POS. LEFT KNEE & RIGHT SHOULDER
A.M.	NORMAL	NORMAL
C.B.	POS. RIGHT DISTAL FEMUR	POS. RIGHT DISTAL FEMUR
A.T.	NORMAL	NORMAL
R.E.B.	POS. L HUMERAL HEAD	POS. L. HUMERAL HEAD OVER BOTH KNEES UNUSUAL PATTERN
T.T.	NORMAL	POS. L HIP
A.D.	BOTH DISTAL FEMORAL SHAFTS POS.	POS. BOTH HIPS AND BOTH DISTAL FEMORAL.

ence. The detailed comparisons are shown in Table 17 and show that J.W.H. is responsible for any preference for MDP.

Table 18 shows the opinions of the same two colleagues about abnormalities seen on these scintigraphs. There is substantial agreement. In only one man (T.T.) is there a difference of opinion as to whether the set is normal or not. Of the 120 polaroid pictures (6 for each man), 13 were commented upon as being abnormal, and the two readers both commented on 7 of these 13 films. The remaining 6 were only commented on by J.W.H. In a critical re-examination of all these films, I am in agreement with all the comments except those on men T.A. and T.T. I would still regard the right shoulder of T.A. as within normal limits and I feel that the mottled increase in isotope uptake in the left femoral head of T.T. is probably urine contamination as it extends caudal to the position of the femoral head itself. Excluding these two films, the other positives are associated with known or suspected caisson disease of bone in R.E.B. and A.D. and some of the others have been commented on above (Section a) - see initials W.B., C.B., and T.A. Diver J.W. is a member of the M.R.C. survey group whose initial scintigraphs were normal but whose scintigraphs at one year follow-up were included as I felt the right humeral head was now abnormal. Both of my colleagues agreed with this. At this time this man had a normal radiographic examination and further follow-up examinations are awaited.

c) SCINTIGRAPHIC FINDINGS IN DIVERS AND COMPRESSED AIR WORKERS WITH SUSPECTED OR DEFINITE OSTEONECROSIS.

Only four of the subjects examined in this group will be discussed. M.McM. This subject was a 45 year old compressed air worker who had had numerous hyperbaric exposures since starting this work when 38 or

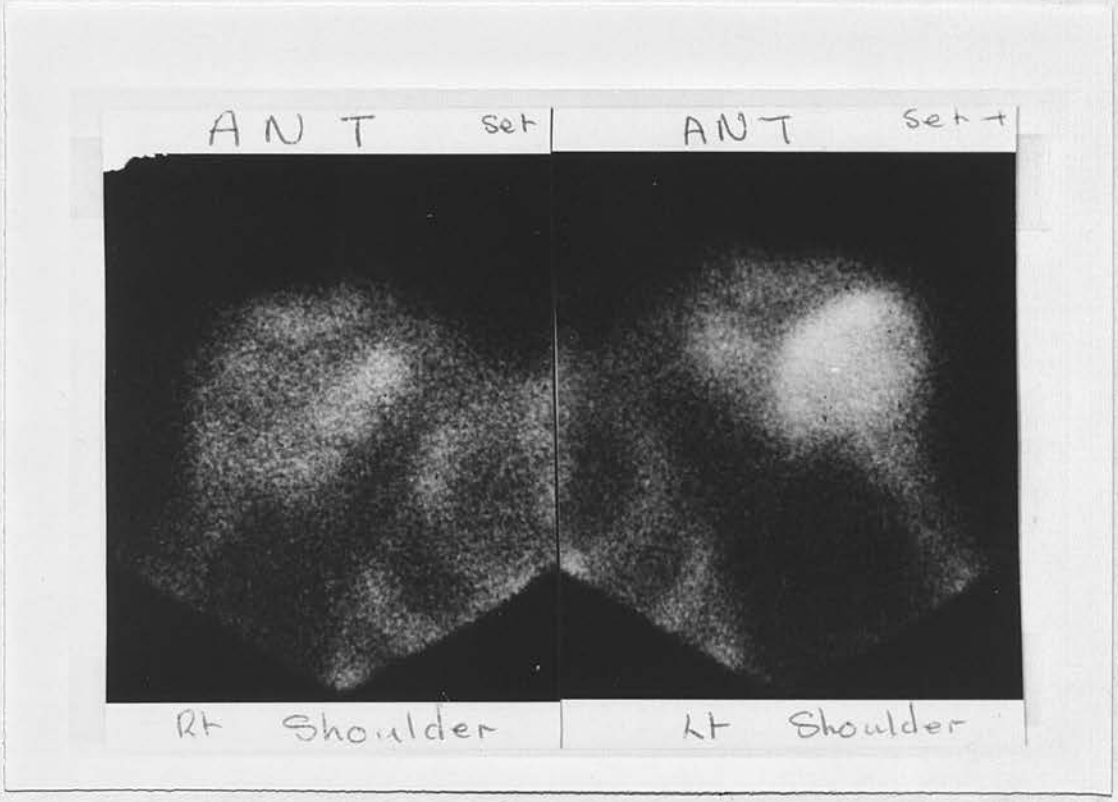


Fig. 51. Scintigraphs of shoulders of subject R.E.B. The whole left humeral head shows a much greater uptake of isotope than the right side.

39 years old. He had noticed discomfort in his left hip for about six weeks, and for a much shorter period occasioned discomfort in his right hip. The left hip tended to give rise to sharp but transient pain on stairs and when he first started walking, and on clinical examination abduction and internal rotation of the left hip were slightly retracted by pain. Radiographic examination suggested that there might be a juxta-articular lesion of the left femoral head. Scintigraphic examination showed a large 'hot spot' of increased uptake of radioisotope corresponding to the area of the whole of the left femoral head. The appearance of the right femoral head was within normal limits, as was the appearance of both shoulders and both knees.

R.E.B. This subject had a long history of caisson disease of bone in multiple sites, certainly for the preceding six years. He had sought advice six years before because a loose fragment from a juxta-articular lesion in his left shoulder had required surgical intervention. The fragment had been pinned back. The crescentic outline of the humeral head articular surface showed a marked increased uptake of radioisotope when scintigraphy was performed six years later. Although this was most marked in a position corresponding to the shoulder joint, the whole humeral head showed a much greater uptake of isotope than that on the scintigraph of the contralateral shoulder (Fig.51). There was also an abnormally increased uptake in the distal femora, more marked on the right side. This man's radiographs showed definite lesions in a number of sites, listed according to the M.R.C. classification as:-

	R	L
Humeral head	A1	A4c
Femoral head	A1	A1. B2
Distal femur	B2	B2
Proximal tibia	B2	B2
Distal tibia	B2	
Distal radius		B1

The last two sites are unusual. Scintigraphy of both wrists was performed but no asymmetry of isotope uptake was detected. This man exemplifies the conclusion of Gregg (1977) that some lesions remain positive when scintigraphy is performed several years later whereas other lesions are not.

R.C. This subject was interesting as he was a diver who had performed air diving on Royal Navy tables from 1943 to 1975 without any problems but had later developed symptoms in his left hip and left knee. Radiographs had indicated a juxta-articular lesion in the left hip and a shaft lesion in the left distal femur. The shaft lesions are usually asymptomatic, but scintigraphy, performed in 1977 showed a 'hot spot' in the medial compartment of the left knee as well as an increased uptake of radioisotope in the left femoral head. The question which was then asked was whether the scintigraph had detected degenerative arthritis of the knee joint (at a stage when radiography was normal) or whether the symptoms and positive scintigraph were associated with the shaft lesion of caisson disease of bone in the distal left femur (which radiographically did not extend to involve the knee joint).

A.D. This 38 year old diver had been diving profesionally for twelve years, his maximum depth being 1000 feet, and his work including much saturation diving in the previous two years. He was asymptomatic, but radiographs showed a suspected B2 shaft lesion in the left distal

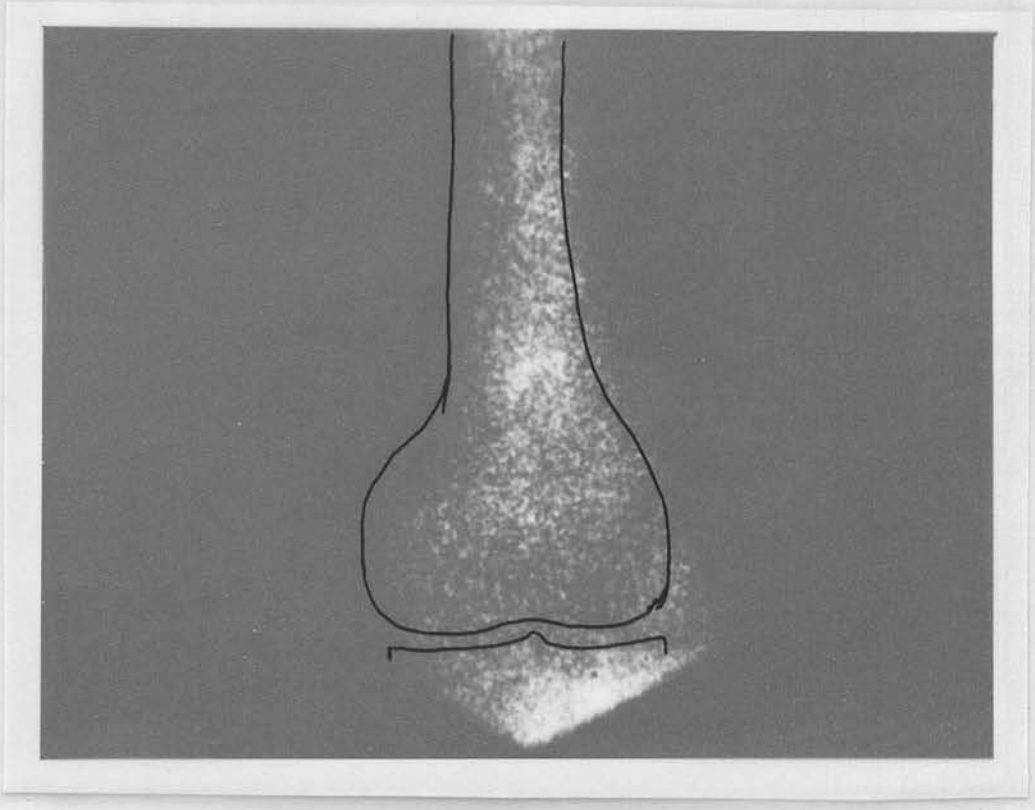


Fig. 52. Scintigraph of left distal femur of subject A.D. showing increased uptake in the distal shaft (see text).

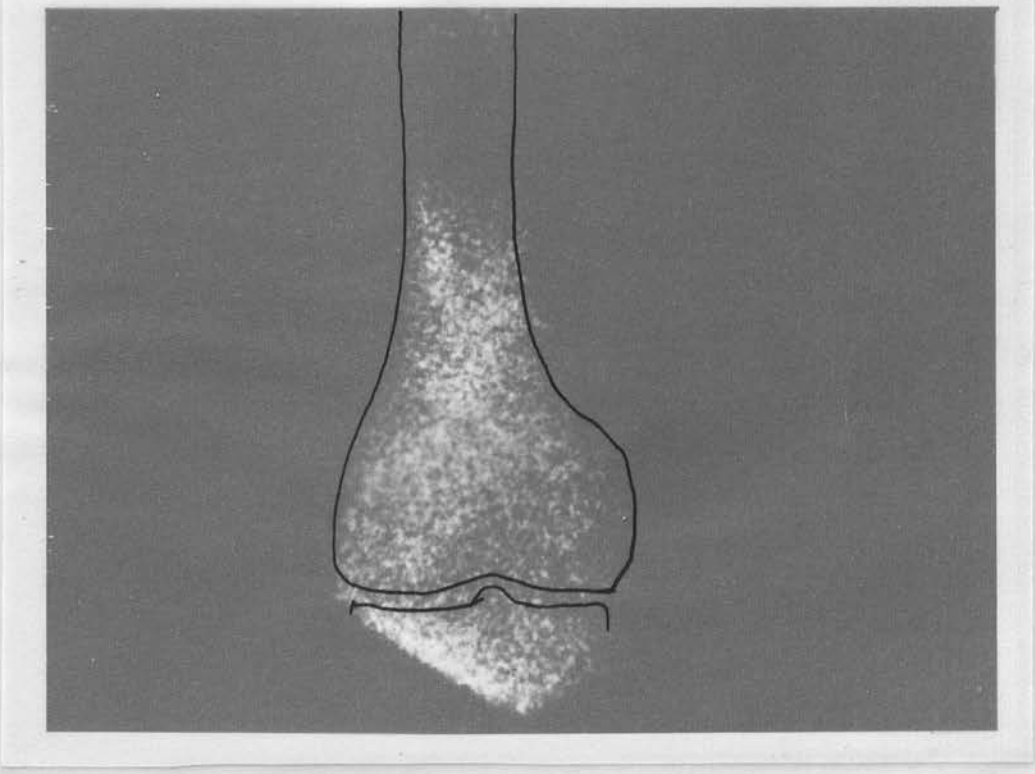


Fig. 53. Scintigraph of right distal femur of subject A.D. (see text).

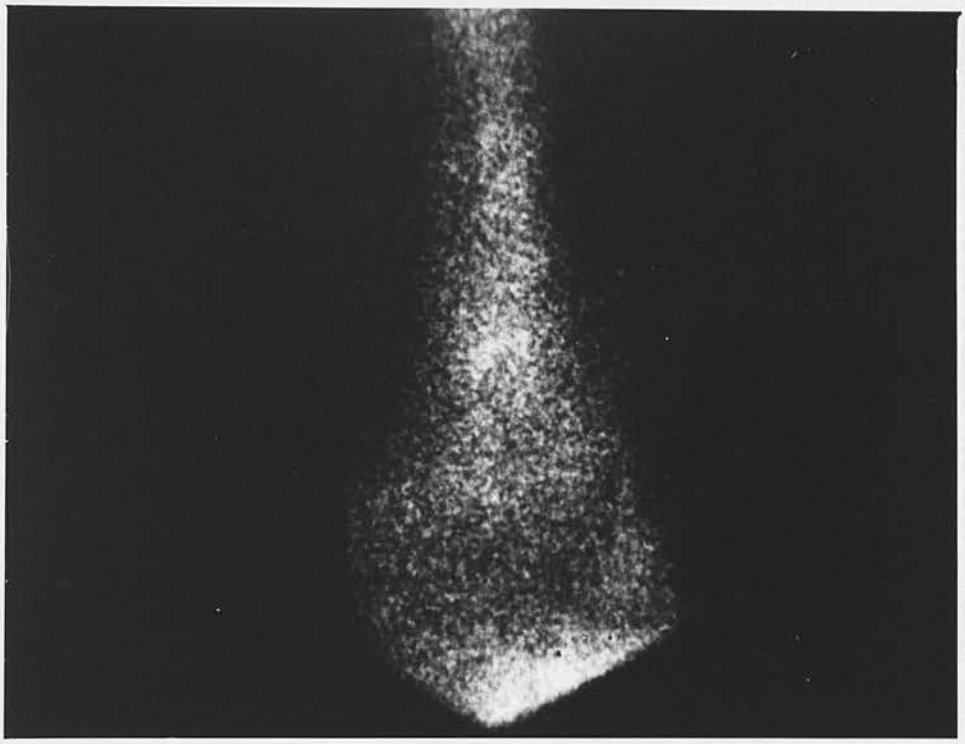


Fig. 52. Scintigraph of left distal femur of subject A.D. showing increased uptake in the distal shaft (see text).

Fig. 53. Scintigraph of right distal femur of subject A.D. (see text).

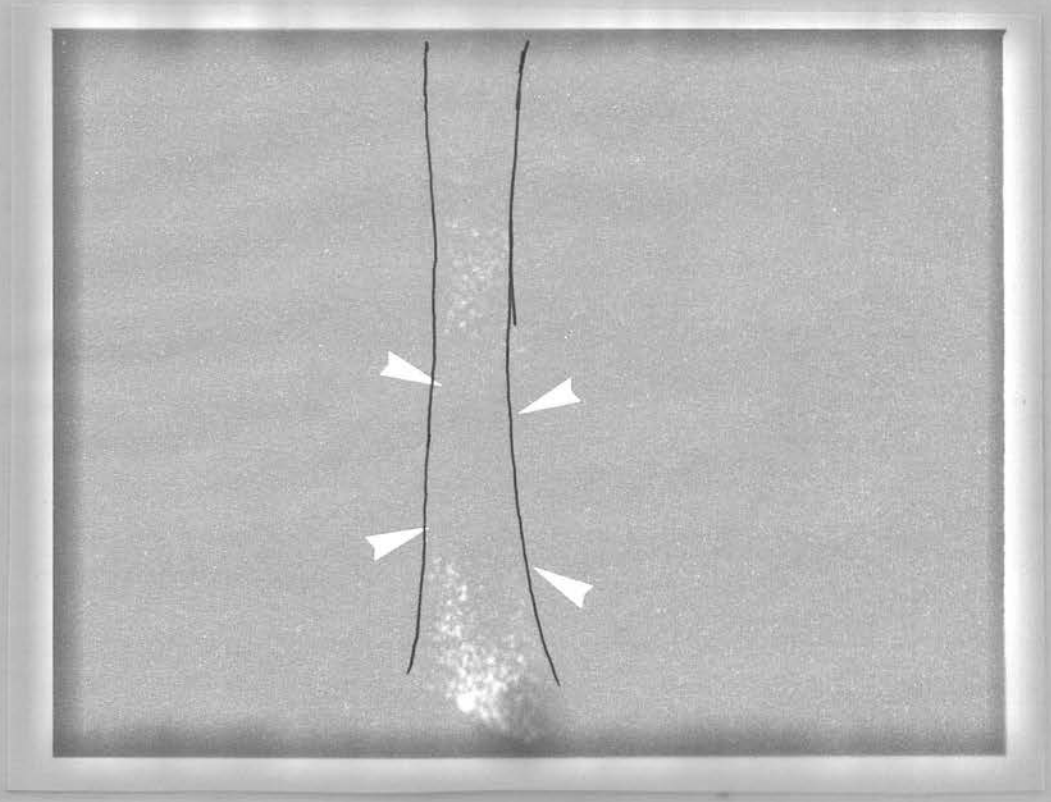


Fig. 54. Scintigraphic appearance of right femoral shaft of subject A.D. The 'cold spot' of decreased uptake (arrowed) is clearly seen. (see text).

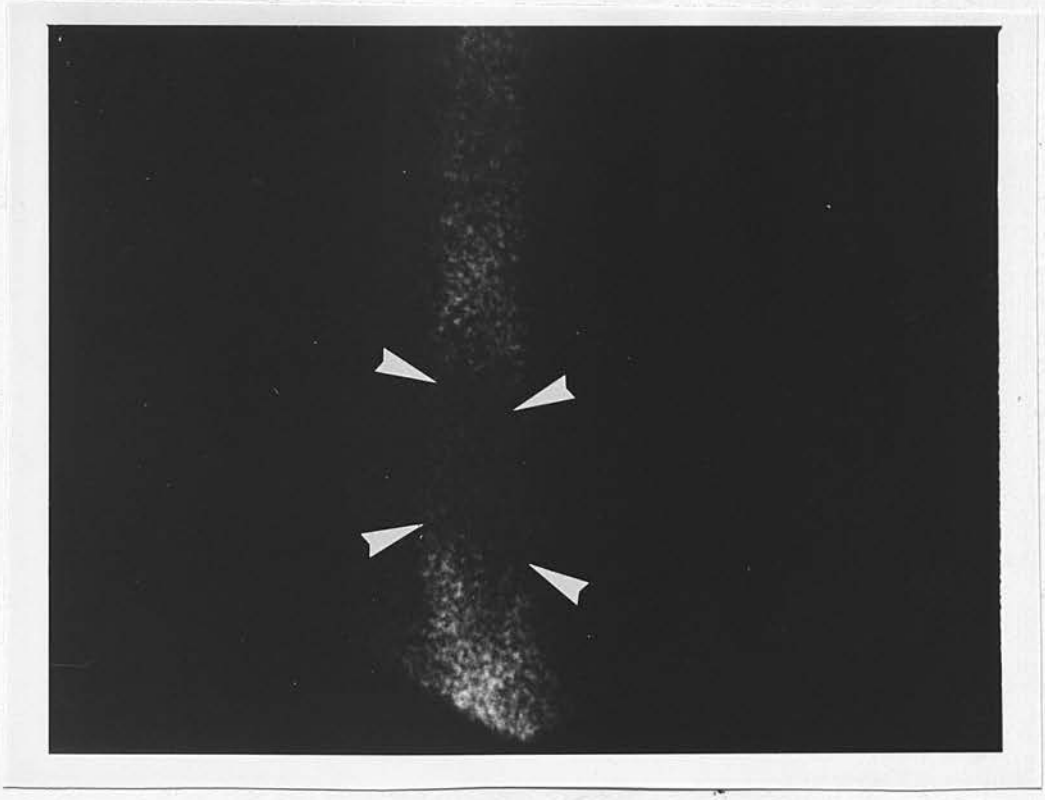


Fig. 54. Scintigraphic appearance of right femoral shaft of subject A.D. The 'cold spot' of decreased uptake (arrowed) is clearly seen. (see text).

femur. Scintigraphy showed an extensive region of increased activity in this area (Fig. 52) but also a rim of increased activity with a central 'cold spot' of decreased radioisotope uptake in the right distal femur (Fig. 53). This is better shown on a special view of the right femoral shaft (Fig. 54) where the area of decreased uptake is clearly seen. As discussed in Chapter 3, a 'cold spot' has never been reported in caisson disease of bone. The fact that we might have been fortunate in detecting a very early lesion was discussed with the referring doctor, who kindly arranged to contact us when the diver was next in this country. This was four months later, and normal diving (including saturation diving) had been allowed during this time. It was expected that the 'cold spot' might have by this time changed to a 'hot spot' (see discussion in Chapter 3) but the appearances were unchanged. This makes this abnormality much more difficult to explain.

DISCUSSION.

None of the men examined experienced any side effects from the intravenous injection of the labelled diphosphonate complex. The dose of radiation is small when compared with that received during conventional radiological examinations. The time taken for scintigraphy is similar to that spent in performing and checking the radiographs required at present for the annual examination for a certificate of fitness to dive. This is so even using a dose of 10 mCi of ^{99m}Tc instead of the usual clinical dose of 15mCi. The smaller dose does not cause any impairment of scintigraph quality.

The objection that scintigraphy is a sensitive but non-specific investigation that would lead to an unacceptably high incidence of unexplained positive results in a normal population seems invalid. Of

the 25 men in the survey group, only two had positive scintigraphs not corresponding to radiographic abnormalities, and in one of these (A.M.) the scintigraphic abnormalities did not correspond to any part of the bony skeleton. In the other man with normal radiographic examination (T.A.) the positive scintigraph corresponded to the medial compartment of a knee joint and might well indicate pathology which will be only detected clinically or radiographically at a later date. The men examined were all active and many were proficient sportsmen and possibly more likely to develop the knee problems seen so often in "contact sports".

I think scintigraphy is therefore suitable as a screening technique for the early diagnosis of caisson disease of bone provided that it does not have an unacceptable incidence of false negative examinations. Because of the time taken for radiographically demonstrable lesions to develop it will probably be some years before this is known. However, only a small incidence of false negatives was found in animal studies of experimental osteonecrosis by Gregg et al.(1977a) and me (Expt. 7) There seems no logical reason why this should not also be true in man. Other workers are performing scintigraphy in high-risk groups but none of the positive scintigraphs have yet shown corresponding radiographic changes (Harrison, 1977).

The findings in the miscellaneous men reported in part c) of the experiment support those of Gregg (1977) that some of the lesions of caisson disease of bone have an abnormally increased uptake of radio-isotope even when they have been present for several years. However, some are negative, and it seems important to perform scintigraphy on men with newly diagnosed caisson disease of bone (as seen radiographically) to see if any recently developed lesions have negative scintigraphs.

From discussions with other workers this does not seem to be the case (King, 1978). The reliability of interpretation of positive scintigraphs of single joints would seem satisfactory from my own experience of independent observers. The comparison of MDP and EHDP as agents for single joint imaging shows that they both produce adequate definition but MDP may be the agent of choice. This is in agreement with the comparison of these agents for whole-body imaging (Subramanian et al.,1975). Another factor in favour of MDP is its faster clearance from the blood stream, allowing scintigraphy to be performed only two hours after its intravenous administration (compared with three hours for EHDP). MDP would be my personal first choice at the present time, but the range of products available is rapidly increasing. I have no experience with the recently described imidodiphosphate complex (Subramanian et al., 1975b).

CONCLUSIONS.

Both MDP and EHDP are satisfactory skeletal imaging agents for single joint scintigraphy using the equipment and protocal described in the experiment.

This study has not so far provided any objections to the use of skeletal scintigraphy for the early detection of caisson disease of bone. There does not appear to be an unacceptable number of false positive scintigraphs despite the non-specificity of scintigraphy for osteonecrotic lesions as opposed to numerous other skeletal abnormalities.

A patient is reported with a scintigraphic "cold spot" in the distal femur probably related to an early lesion of caisson disease of bone.

SECTION 3

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DISCUSSION.

CHAPTER 8. GENERAL DISCUSSION AND CONCLUSIONS.

DISCUSSION.

The aim of this study was to investigate the aetiology and early diagnosis of caisson disease of bone, with special reference to any factors which might influence the management of juxta-articular lesions before symptoms develop. The work was part of an ongoing study within the Medical Research Council Decompression Sickness Research Team at Newcastle-upon-Tyne. Work had already been performed to develop suitable animal models, both using artificial microemboli (in rabbits) and hyperbaric exposures (in miniature swine). The hypothesis inherent in much of the experimental work was that gas bubbles were an important aetiological factor. This hypothesis is also basic to this thesis and it requires emphasis before any discussion that this remains a hypothesis and not a fact. The difficulty of devising experiments to test this hypothesis has already been outlined in Chapter 2.

Several experiments had already been performed to investigate the involvement of some of the aetiological factors other than bubble emboli postulated as causing vascular obstruction. For example, Cox (1974) had foamed blood *in vitro* with streams of bubbles and looked for lipid granules afterwards. None were found, but the scheme proposed by Philp *et al.* (1971), shown in Fig 1a, is not negated by this experiment because the scheme postulates catecholamine release as a necessary factor *in vivo*. Even more important is the fact that *in vitro* experiments do not allow for endothelial damage by bubble surfaces releasing chemicals locally (e.g. tissue thromboplastin). I could not think of any way of performing a similar sort of experiment *in vivo* without the

animal dying from aeroembolism. I tried to investigate the involvement of fibrinogen *in vivo* as this can be easily labelled radioisotopically, but the experiment was technically unsuccessful. This is a difficult hypothesis to investigate, and I find it of interest that my successor in the research team is investigating the role of extravascular factors such as fluid shifts into fat cells with hyperbaric exposure. This seems a sensible approach as intravascular factors alone cannot account for the distribution of lesions found clinically. This is deduced from the blood flow distribution studies of the femur using labelled microspheres reported by Gregg (1977). The observed distribution of shaft lesions in the femora of men affected with caisson disease of bone is that the great majority of the lesions are in the distal shaft. This has not been explained by simple environmental factors (Decompression Sickness Panel of Medical Research Council, 1971), and blood flow studies in the rabbit (Gregg, 1977) and in the dog (Bouteiller *et al.*, 1978), have not shown a decreased vascularity of the distal compared with the proximal femoral shaft.

One aspect which did not depend on an embolic hypothesis was investigated in Experiment 1. This was designed to see if any extraneous factors were necessary for the conversion of an area of anoxic marrow within the medullary cavity of the femur to a lesion similar to the shaft lesion of caisson disease of bone. The hypothesis was made that this would occur *in vivo* as part of a natural body repair process. Marrow was rendered anoxic as described and the results supported the hypothesis. The experiment was performed in rabbits and there was some indication that within three months the lesions tended to be undetectable and to have possibly repaired. This time course would correspond to that found in the avascular rabbit femoral

head (Szepesi, 1978). Two questions then arise. The first is whether areas of anoxic marrow in humans occur which completely repair themselves. This cannot strictly be answered at present. No lesions large enough to be demonstrated radiographically have been observed to resolve, but post mortem material has shown that large areas appear to have repaired. Size may be important and the lesions which completely repair may be too small to ever be demonstrated by conventional radiography. The second question is that if lesions completely repair in the rabbit, why do they stop repairing in man? The first is a simple size phenomenon which might also explain the difficulty of producing lesions in animals following hyperbaric exposure. The second is similar and emphasises the limitation of new bone formation because of decreased oxygen tension at the limits of the repair. I cannot discover any experimental evidence to support this suggestion. A mechanical hypothesis was suggested by Weatherley et al. (1977) and this may be appropriate for juxta-articular lesions. The shaft lesions occur predominantly within the marrow cavity and load-bearing would not occur in this position nor account for the observed configuration of the bony shell within the boundary zone of these lesions. A novel explanation is suggested in the discussion of Experiment 1; that living marrow is osteogenic and attempts to surround itself with bone, and that the presence of an area of anoxic marrow creates a surface of living marrow which is the stimulus for the repair process and the formation of the observed boundary zone.

This would mean that fewer aetiological factors might need to be postulated in the aetiology of caisson disease of bone than indicated, for example, in Fig. 1a. Any factor which caused an interruption in the blood supply to an area of marrow long enough to cause it to become anoxic or necrotic sufficiently to create a boundary of living marrow

could lead to a lesion resembling that seen in caisson disease of bone (and incidentally a lesion like that seen in sickle cell disease). This might be intravascular or extravascular factors or a combination of both. The experiment does not permit any estimation of the duration of ischaemia necessary to damage the marrow and it is therefore not appropriate here to add to the speculation of what these factors might be. It may be that there is no need for any permanent interruption of blood supply as marrow seems more sensitive to ischaemia than bone and the duration of ischaemia necessary to produce osteocyte death is probably 6 - 12 hours. It might be useful to repeat the experiment of Kenzora (1972) on marrow cells as opposed to osteocytes.

The problems of achieving earlier diagnosis of caisson disease of bone are outlined in Chapter 3. A promising line of research in this field was already in progress. It appeared that the miniature pig might be a suitable animal model for the development of osteonecrosis following hyperbaric exposures and it also appeared that urinary hydroxyproline excretion, serum ferritin estimation, and skeletal scintigraphy might be useful parameters to study further.

The main body of the experimental work was designed to measure all these parameters as extensively as possible in 6 minipigs undergoing multiple hyperbaric exposures with the same decompression profile as used on compressed air work contracts (and successfully used in minipigs by Gregg (1977)). The limit of 6 minipigs was determined by the animal house space available. No control animals were used but I was personally present at the post-mortem of the two control animals studied and reported by Gregg and studied the sectioned bones macroscopically and microscopically from these animals. The scintigraphs and radio-

graphs of these animals were particularly valuable in providing a baseline of normal appearances. It is, therefore, emphasised, for example, that the prominent 'spurs' seen endosteally extending into the marrow cavity in the humerus radiographs (Figs. 24 and 25) are a normal appearance and not related to hyperbaric exposure.

In addition to the parameters already studied by the research team I decided to study collagenolytic enzymes measured in serum as proline imino peptidase activity (serum PIP). Experiment 2 showed that the use of this assay failed to detect areas of artifically induced bone and marrow necrosis. Of the other parameters, serum ferritin proved a problem. It was known to be a difficult assay and the haematologist who had previously performed the assay in Newcastle-upon-Tyne had only worked with rabbit and human serum and not that of minipigs and had left to work overseas. The assay could be obtained on human serum samples on a commercial basis but not on minipig serum. A research worker has now been employed for two years developing this assay but no results are available. This is not as disappointing as it might have been because none of the 6 minipigs studied in Experiment 3 to 6 developed bone or marrow necrosis. All the parameters studied (24 hour total urinary hydroxyproline excretion, serum proline-imino-peptidase activity, skeletal scintigraphy, and radiography) showed no abnormality except for the increased urinary hydroxyproline excretion in minipig Tom, which remains unexplained.

These parameters are also being studied in human volunteers, especially during dives conducted by the Admiralty Marine Technology Establishment in Portsmouth. Skeletal scintigraphy is provoking the most interest and is being studied in several centres. It seemed important to undertake a prospective study of this technique in a

group of men 'at risk' of developing caisson disease of bone and the setting up of this study is described in Experiment 9. Despite the numerous skeletal lesions that have been listed as showing an increased uptake of diphosphonate imaging agents, there did not seem to be an unacceptable number of false positive scintigraphs and the technique may well prove valuable in the earlier diagnosis of caisson disease of bone. In contrast to the biochemical studies under consideration, skeletal scintigraphy has the advantage that it also indicates the site of a suspicious positive result, though as indicated in Experiment 8, there is still disagreement about the exact pathophysiological process involved.

Experiment 8 presents a technique and preliminary results only. Further work is in progress to study time intervals shortly after injection of labelled diphosphonate to try and confirm whether the bone cells have a role in localising its uptake.

Management of patients with caisson disease of bone depends on the site of the lesion and the presence or absence of symptoms. Shaft lesions do not cause symptoms. Men with such lesions might be barred from further hyperbaric exposure, but there is no statistically significant evidence that if they continue to undergo hyperbaric exposure they have any greater probability of developing a juxta-articular lesion than men without a shaft lesion (Trowbridge *et al.*, 1979). At present such a policy would therefore be unwarranted.

Juxta-articular lesions causing symptoms may prevent the physical efforts required in the work of divers and compressed air workers. A painful shoulder or hip joint in a young otherwise fit man may require

arthrodesis (or possibly arthroplasty in the hip joint). If the lesion is discovered before symptoms arise, is there anything that can be done to prevent later problems? The natural history of such a lesion is variable; it may rapidly progress to structural failure of the joint or remain unchanged for many years. Any treatment must therefore have a minimal risk attached to the treatment itself.

As a result of the hypothesis developed in the discussion following Experiment 1, I would suggest that it might be valuable to destroy an established junctional zone seen radiographically (when the repair process has ceased) to recreate a surface of living marrow not surrounded by bone and to allow the repair to proceed. This is not a new idea. It was suggested by Phemister (1920) to allow 'revascularisation'. It has been performed fairly extensively using a bone graft inserted into a hole across the boundary zone, most recently with a muscle pedicle (Boettcher et al., 1970). However, others have performed a 'forage-biopsy' on the avascular femoral head and claimed good results in cases of symptomatic idiopathic ischaemic necrosis of the femoral head in adults (Arlet and Ficat, 1965). It may be that the making of the hole is more important than the insertion of the graft. If any doctor was therefore inclined to offer treatment to asymptomatic cases this may be a valuable thought, because it is far less of a procedure to perform a forage biopsy under biplane radiographic control than to perform a muscle pedicle bone graft. However, having said this, there is no evidence at present to merit any surgical intervention in asymptomatic juxta-articular lesions.

It might therefore be asked what point there is in striving for earlier diagnosis. If the potentially disabling juxta-articular lesion can be discovered (e.g. by skeletal scintigraphy) before the

junctional zone has formed, might there be some way of stimulating the repair process to continue. The obvious suggestion, if the lesion is basically ischaemic, would be maximal oxygenation, presumably by a course of hyperbaric oxygen treatments. This is not necessarily logical, however, because hyperbaric oxygen may cause vasoconstriction (Bassett and Bennett, 1977) and more damage. Furthermore there is no evidence that it is lack of oxygenation which causes the repair process to cease.

More study is therefore required before any suggestions can be made regarding management of an early case of a juxta-articular lesion. Post-mortem material from diving accidents is one possible source of further information as many of our ideas of the sequence of the repair process is at present extrapolated mainly from studies on post-traumatic avascular necrosis. Information might also be obtained from animal studies, although the minipig does not seem to be a reliable animal for developing osteonecrosis before repeated hyperbaric exposures become difficult because of an increasing frequency of acute decompression sickness. This has limited the laboratory studies to total exposure times of around 300 hours and compressed air workers with this short length of exposure do not have a particularly high incidence of osteonecrosis. Figures extracted from the Medical Research Council Decompression Sickness Central Registry indicate that there may be an increased incidence of caisson disease of bone in divers who have performed saturation dives and it may be that the use of saturation dive profiles in dry chambers on experimental animals such as miniature swine might be useful.

In any such research, the techniques described in this thesis may prove useful, especially the indwelling catheters used in Experiment 5, which had surprisingly few technical problems. Skeletal

scintigraphy appears to be a promising technique for the earlier detection of caisson disease of bone, but the results of the prospective study described in Experiment 9 may take several years to confirm this.

CONCLUSIONS.

1. The aetiology of caisson disease of bone is incompletely understood. Re-implantation of autologous marrow rendered anoxic for two hours into rabbit femora produced a lesion of abnormal marrow with a surrounding ring of fibrous tissue and bone in five out of twelve experiments. This may mean that any factor causing an area of marrow anoxia or necrosis could lead to the development of a lesion resembling that seen in caisson disease of bone.

2. Collagenolytic enzymes, measured as the serum proline imino peptidase activity, showed no significant change in nine rabbits in the fourteen days following an intra-arterial injection of microspheres, even though bone and marrow necrosis developed in all nine animals.

   Serum proline imino peptidase activity will probably therefore not be useful in attempts at earlier detection of lesions of caisson disease of bone in man.

3. Six castrated male Göttingen miniature swine were subjected to repeated hyperbaric exposures (range 35 - 84) of 27 p.s.i.g., usually for six hours, with a standard decompression. Although much acute decompression sickness resulted, no lesions resembling caisson disease of bone were found at autopsy six weeks after the last decompression. However, two of these six minipigs had aeroembolism of the marrow cavities of long bones at autopsy.

   Indwelling silastic right atrial catheters can be maintained in un-

restrained miniature swine for up to twelve weeks with daily checks of their patency.

4. The mean total urinary hydroxyproline excretion of six castrated male Göttingen miniature swine aged 12 months was 27.5 $\pm$ 14.2 mg/24h. There was no evidence of an increase in total urinary hydroxyproline excretion in 5 out of 6 minipigs following repeated hyperbaric exposures, and the inaccuracies inherent in attempting 24 hour urine collections in uncatherised large animals made any abnormal measurements difficult to interpret.

   No change was detected (compared with pre-exposure values) in the serum proline imino peptidase activity of six miniature swine following multiple hyperbaric exposures and several episodes of acute decompression sickness.

5. A technique is described for skeletal scintigraphy and radiography of the humeri and femora of miniature swine.

   An earlier report that skeletal scintigraphy performed following experimental induction of osteonecrosis in the femora of adult New Zealand White rabbits shows an increased uptake in the majority of areas of osteonecrosis is confirmed.

6. A technique is described for the rapid preparation of thin sections of undecalcified cancellous bone with a surface smooth enough for autoradiography.

   Early results of autoradiographic studies indicate that the enhanced concentration of skeletal imaging agents in areas of new bone formation may be a result of an increase in their uptake by the bone cells.

7. Single joint scintigraphy of the hips, shoulders, and knees of human subjects is reported. Using the equipment and protocol described, both diphosphonate skeletal imaging agents studied (MDP and EHDP)

appeared satisfactory.

Skeletal scintigraphy may be a useful technique for the earlier detection of caisson disease of bone. Specifically, there did not appear to be an unacceptable number of false positive scintigraphs in the population studied (commercial divers aged 20-30 years).

The setting-up of a prospective study of skeletal scintigraphy in 25 men starting a diving career is described.

A patient is reported with a scintigraphic "cold spot" in the distal femur probably related to an early lesion of caisson disease of bone.

8. A hypothesis is advanced that the cessation of repair of lesions of caisson disease of bone may be because the surrounding living marrow succeeds in surrounding itself with bone.

If this hypothesis is accepted, a recommendation should be made to reconsider the role of forâge-biopsy in any surgical management of juxta-articular lesions of caisson disease of bone.

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DECLARATION.

I, John Stothard, declare that this thesis has been composed by myself. I also declare that all the experimental work in it has been performed by me except as noted in the acknowledgements.

J. Stothard.

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The following experiments have had results presented to scientific meetings and abstracts have been published as indicated:-

Expt. 1. Presented in part to the annual scientific meeting of the Undersea Medical Society, Seattle, April 1978.
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